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GENETICS

A Periodical Record of Investigations Bearing on Heredity and Variation

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STUDIES ON THE VIGOR OF SILKWORMS, *BOMBYX MORI* L.

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Received June 4, 1929

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INTRODUCTION

With the aim of investigating the cause of the alternation of voltinism, which has not yet been cleared up, I have already tried the ovarian transplantation between univoltine and bivoltine larvae and have found that the eggs laid from the transplanted ovaries are of the same nature in voltinism as the eggs laid by the host from its ovaries, that is, the phenomenon is conditioned by the nature of the blood of the foster mother, and this made it clear that, as the apparent matrocliny in the inheritance of voltinism is due only to the physiological influence of the foster mother, the term "maternal inheritance" employed by TOYAMA can not be correctly applied to the inheritance of voltinism. On the other hand, it was further observed that the voltinism, as well as all the other Mendelian characters, that is, the markings of larvae, the color of cocoons and so forth, of offspring (next generation) produced from the transplanted ovaries, were not at all influenced, there being nothing to show such an effect of the foster mother as GUTHRIE (1908) observed in similar experiments made with chickens.

New experiments, moreover, showed that the individuals reared from the eggs of transplanted ovaries have always higher vigor than those of the control, when the operation of ovarian transplantation is successfully

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carried out. If the embryos developed out of the transplanted ovaries placed in different conditions are thus influenced, then it must be expected that the same effect will be seen in the crossing between two variants produced from one and the same breed under different environments. With this point of view, I have made further experiments in which I have also recognized the greater vigor of the crossing between hibernating (annual) and non-hibernating (biannual) variants produced from a bivoltine breed under different conditions, as compared with the original inbred strain. It is believed that these new experiments are not only practically important but that they also may throw some light on the cause of the vigor due to hybridization.

Before proceeding further, I should like to take this opportunity to tender my hearty thanks to Doctor C. ISIKAWA, for his valuable advice during the course of my present experiments. Thanks are due to Professor E. G. CONKLIN for his favor in looking through the manuscript.

VIGOR OF SILKWORMS PRODUCED FROM OVARIAN TRANSPLANTATION BETWEEN DIFFERENT BREEDS

Material and Method

As I have already stated in my previous report (1926) the technique of ovarian transplantation, I need not dwell on it here. To investigate the influence upon the vigor of the offspring resulting from the transplanted ovaries is more difficult than to observe directly the alternation of voltinism of the eggs from transplanted ovaries; because, in the former case, the host must be fully developed, more pains must be taken to prevent bleeding, and the offspring resulting from the transplant has to be well grown without suffering from the bad influence of the host.

For the purpose of making ovarian transplantation, the larvae of an early instar may be better, but it is not only impossible to operate on such very young larvae, but also impossible to use the largest fully-developed larvae of the last instar, because of the silk glands being squeezed out from the slit so that the operation cannot be carried out. Accordingly, the larvae at the beginning of the fifth instar (first and second day after the fourth moulting) were largely used in this case. As a matter of fact, the primordial egg-cells in the ovaries of larvae in such a stage are still so small that each contains only the nucleus and very little cytoplasm, but after the larvae have developed for about a week, the eggs rapidly enlarge, filling up with a large quantity of nutrients and cytoplasm, as reported by MATIDA (1926). Consequently, the larvae at the beginning of the fifth instar

are not unfavorable material for the purpose of investigating the physiological influence of the foster mother on the transplanted ovary.

Into the bodies of the pure breed, *Jap. No. 107*, a strong bivoltine race, from which the ovaries were removed, the ovaries of two other breeds were transplanted; one was the worm I termed cy strain, the reproductivity of which was extremely lowered by successive inbreeding since I first selected it in 1918, and the other the pure breed, *Jap. No. 105*, which showed no bad effect whatever of inbreeding for many years at the IMPERIAL SERICULTURAL EXPERIMENT STATION OF TOKYO.

Experimental results

In the experiments on ovarian transplantation we have as a rare occurrence a portion of the ovarian string duct remaining in the body of the host from which the ovary was removed which is connected with a point of the small part of the duct of the transplanted ovary, thereby forming an oviduct so favorably and completely connected with the ovarian string duct of the host that its development appeared normal, except that its four ovarian tubules were shorter in length, sometimes fewer in number and rarely contained any eggs. Accordingly, the eggs laid from the transplanted ovary in most cases were few in number. Into the bodies of the pure breed, *Jap. No. 107*, a bivoltine race, from which the ovaries were removed, the ovaries of the cy strain were transplanted. The results obtained from this experiment are given in table 1.

Table 1 indicates that the worms experimented upon showed increased weight and reproductiveness in spite of the shorter duration of larval life, as compared with those of the control. It is worth while to mention that the number of eggs in the experimented worms showed an increase by about 20 percent over the controls. Subsequently, into the bodies of the same breed as above described, the ovaries of the pure breed, *Jap. No. 105*, which showed no bad effects of inbreeding, were transplanted. These experimental results are given in table 2.

In the second table also, a tendency similar to that shown in table 1 is recognized, that is, the experimented worms showed greater vigor than the controls, though they showed a somewhat less degree of increased vigor than the cy strain showed. It is, however, noteworthy that the worms reared from the ovaries of a strain (table 1, cy worms) greatly weakened by successive inbreeding showed more strongly the influence of the host than did the pure breed (table 2, *Jap, No. 105* worms) which showed no bad effects of previous inbreeding.

Moreover, it is necessary to ascertain whether such influence of the fos-

TABLE 1

♀ (*Host—Jap. No. 107←Ovaries—cy*) × ♂ *cy.*

	SERIAL NUMBER	WEIGHT OF EACH WORM		TIME OF FEEDING DAYS—HOURS	NUMBER OF MOTHS	NUMBER OF EGGS			AVERAGE NUMBER OF EGGS	
		AFTER 3RD MOULTING GRAMS	AFTER 4TH MOULTING GRAMS			LAID BY ALL MOTHS	REMAINING IN BODIES	TOTAL	PRODUCED BY EACH MOTH	LAID BY EACH MOTH
Experiment	32	0.103	0.485	30– 3	6	3026	429	3455	575.83	504.33
	33	0.103	0.542	30–12	13	5906	775	6681	513.92	454.31
	34	0.100	0.482	30– 6	11	4943	503	5446	495.09	449.36
	35	0.090	0.454	30–12	9	3957	381	4338	482.00	439.67
		(Average)	(Average)	(Average)	(Total)	(Total)	(Total)	(Total)	(Average)	(Average)
		0.099	0.491	30– 8	39	17832	2088	19920	513.462	457.692
						(Standard deviation)			±63.510	±61.277
Control	37	0.098	0.472	30–12	21	7340	1163	8503	404.90	349.52
	38	0.085	0.410	30–12	10	4091	414	4505	450.50	409.10
	39	0.087	0.418	30–12	10	3762	691	4453	445.30	376.20
		(Average)	(Average)	(Average)	(Total)	(Total)	(Total)	(Total)	(Average)	(Average)
		0.090	0.434	30–12	41	15193	2268	17461	428.049	370.732
						(Standard deviation)			±72.456	±76.887

TABLE 2

♀ (*Host—Jap. No. 107*←*Ovaries—Jap. No. 105*) × ♂ *Jap. No. 105.*

	SERIAL NUMBER	WEIGHT OF EACH WORM		TIME OF FEEDING DAYS—HOURS	NUMBER OF MOTHS	NUMBER OF EGGS			AVERAGE NUMBER OF EGGS	
		AFTER 3RD MOULTING GRAMS	AFTER 4TH MOULTING GRAMS			LAID BY ALL MOTHS	REMAINING IN BODIES	TOTAL	PRODUCED BY EACH MOTH	LAID BY EACH MOTH
Experiment	2	0.154	0.928	31–19	16	7236	746	7982	498.88	452.25
	3	0.157	0.908	31– 0	10	4999	584	5583	558.30	499.90
	4	0.147	0.833	31– 0	12	5994	644	6638	553.17	499.50
	6	0.167	0.843	32– 0	34	16143	1319	17462	513.59	474.80
	7	0.162	0.850	31– 6	13	6138	431	6569	505.31	472.15
	11	0.167	0.780	32–12	63	29560	2566	32126	509.94	469.20
	15	0.159	0.792	32– 4	15	6930	762	7692	512.80	462.00
	16	0.157	0.748	32–12	21	10273	1291	11564	550.67	489.19
	19	0.152	0.795	32– 0	17	7735	704	8439	496.41	455.00
		(Average) 0.158	(Average) 0.830	(Average) 31–19	(Total) 201	(Total) 95008	(Total) 9047	(Total) 104055	(Average) 519.527	(Average) 473.383
						(Standard deviation)			±60.119	±57.035
Control	21	0.140	0.741	32– 0	60	27179	1255	28434	473.90	452.98
	22	0.150	0.672	32–12	70	31617	1703	33320	576.00	451.67
	23	0.134	0.698	31–10	50	22693	1360	24053	481.06	453.86
	24	0.162	0.735	31–19	49	19781	1890	21671	442.27	403.69
	25	0.160	0.780	32–12	36	15293	1469	16762	465.61	424.86
		(Average) 0.149	(Average) 0.725	(Average) 32– 1	(Total) 265	(Total) 116563	(Total) 7677	(Total) 124240	(Average) 468.019	(Average) 440.094
						(Standard deviation)			±74.188	±77.355

ter mother upon the offspring could be carried over to succeeding generations. For that purpose, the vigorous lines of worms in the above two tables were selected out of those thus made vigorous, and were reared in the autumn to compare with the worms of the original line. The results obtained were as follows:

TABLE 3

Rearing results of the next generation of the cy strain used in the experiment of table 1.

	SERIAL NUMBER	WEIGHT OF EACH WORM AFTER 4TH MOULTING (GRAMS)	TIME OF FEEDING (DAYS–HOURS)	NUMBER OF MOTHS	NUMBER OF EGGS: LAID BY ALL MOTHS	NUMBER OF EGGS: LAID BY EACH MOTH
Experiment	140	0.392	24–23	11	3641	331.00
	141	0.366	26–15	8	2828	353.50
	142	0.367	26–21	8	2629	328.63
	144	0.390	24–21	20	6405	320.25
	146	0.360	24–15	10	2844	284.40
	147	0.343	24–23	17	5643	331.94
		(Average) 0.370	(Average) 25–12	(Total) 74	(Total) 23990	(Average) 324.324
				Standard deviation ± 64.981		
Control	151	0.356	26–23	8	2529	316.13
	152	0.389	24–23	8	2547	318.38
	153	0.360	26–21	10	3016	301.60
		(Average) 0.368	(Average) 26– 6	(Total) 26	(Total) 8092	(Average) 309.615
				Standard deviation ± 66.534		

In table 3, further influence on the next generation was recognized, though in a less degree. On the other hand, table 4 showed no trace whatever of any influence exerted on the second generation by the original transplantation.

VIGOR OF SILKWORMS OBTAINED FROM THE CROSSING OF TWO VARIANTS BRED UNDER DIFFERENT ENVIRONMENTS

The results obtained from the foregoing experiments led me to try again the following experiment. From the eggs of a bivoltine breed, worms of 2 groups were hatched out by subjecting them to the conditions induced by different environments; 1 was made completely univoltine by the application of a high temperature of over 25°C during the incubation of the em-

bryonal stages, while the other became bivoltine under a temperature less than 15°C. As I have already reported, we ascertained that the alternation of voltinism was caused by the local condition, and each individual bearing different voltinism did not contain the same blood as regards the determination of voltinism, that is, the univoltine individual tended to secrete the voltinism-determiner (so-called inhibitory substance of WATANABE) from the post-embryonal somatic cells by the stimulus of a high tem-

TABLE 4

Rearing results of the next generation of the breed Jap. No. 105 used in the experiment of table 1.

	SERIAL NUMBER	WEIGHT OF EACH WORM AFTER 4TH MOULTING (GRAMS)	TIME OF FEEDING DAYS–HOURS	NUMBER OF MOTHS	NUMBER OF EGGS LAID BY ALL MOTH	NUMBER OF EGGS LAID BY EACH MOTH
Experiment	130	0.664	25–20	27	9559	354.04
	132	0.658	25–20	16	6704	419.00
	136	0.664	25–20	27	10902	403.77
	137	0.632	25–20	30	12276	409.20
		(Average) 0.655	(Average) 25–20	(Total) 100	(Total) 39441	(Average) 394.250
				Standard deviation ±64.600		
Control	117	0.645	25–20	35	13976	399.31
	118	0.682	25–20	26	10205	392.50
	119	0.661	25–20	16	6441	402.56
	120	0.681	25–20	42	16730	398.33
		(Average) 0.667	(Average) 25–20	(Total) 119	(Total) 47352	(Average) 398.529
				Standard deviation ±69.981		

perature and to transmit it into egg-cells in their maturation, thus causing them to hibernate, while the bivoltine individuals which developed at a low temperature did not secrete such a substance and consequently their eggs did not hibernate. It was interesting to note from the foregoing experiments the results of rearing worms obtained from the crossing of 2 such variants produced from one and the same breed under different temperatures, as compared with the pure original breed.

Material and method

One batch of eggs laid by a moth of the pure bivoltine race was divided into 2 equal parts; 1 was incubated and subjected to the high temperature

(25°C) in order to produce the moths laying hibernating eggs, the other to the low temperature (15°C) to make the moths lay non-hibernating eggs. Thus, these 2 groups of worms were obtained for the purpose of breeding one with the other reciprocally. The hibernating eggs were hatched out artificially in the autumn for the purpose of rearing them together with the non-hibernating eggs which were hatched out naturally. The material used for these experiments was the pure bivoltine breed, *Jap. No. 105*.

Experimental results

The rearing results obtained from the crossing of 2 variants bred under different environments, as above stated, will be shown in the following 2 tables.

TABLE 5

Rearing results of worms hatched artificially from hibernating eggs.

	SERIAL NUMBER	WEIGHT OF EACH WORM AFTER 4TH MOULTING (GRAMS)	TIME OF FEEDING DAYS–HOURS	NUMBER OF MOTHS	NUMBER OF EGGS LAID BY ALL MOTHS	NUMBER OF EGGS LAID BY EACH MOTH
Experiment ♀hibernating ×♂non-hibernating	109	0.712	24–19	43	20391	474.21
	110	0.772	24–23	130	60528	465.60
	111	0.784	24–23	111	51452	463.55
	112	0.662	25–19	127	56144	442.08
		(Average) 0.733	(Average) 25– 3	(Total) 411	(Total) 188515	(Average) 459.124
				Standard deviation ±61.920		
Control ♀ hibernating × ♂hibernating	101	0.713	25–14	150	61520	410.13
	102	0.715	24–22	127	57816	455.24
	103	0.730	25– 0	103	45087	437.74
	104	0.743	24–23	119	52201	438.66
		(Average) 0.725	(Average) 25– 3	(Total) 499	(Total) 216624	(Average) 434.369
				Standard deviation ±72.658		

In these two tables also, the crossings between 2 characters produced under different environments showed a shorter duration of larval life, a somewhat greater weight and higher reproductivity, as I presumed, thus showing higher vigor as the previous experiments indicated.

The sericulturist knows by experience that when 2 variants of univolting

and bivoltine worms are produced from a bivoltine breed and reared at the same time in the autumn, the latter should show a somewhat greater vigor than the former. In this case also, the rearing results of the control in table 6 (bivoltine worms) showed a somewhat greater vigor than those of the control in table 5 (univoltine worms). These results were very likely due

TABLE 6

Rearing results of worms hatched naturally from non-hibernating eggs.

	SERIAL NUMBER	WEIGHT OF EACH WORM AFTER 4TH MOULTING (GRAMS)	TIME OF FEEDING DAYS–HOURS	NUMBER OF MOTHS	NUMBER OF EGGS LAID BY ALL MOTHS	NUMBER OF EGGS LAID BY EACH MOTH
Experiment ♀ non-hibernating ♂hibernating	113	0.715	24–19	103	48082	466.82
	114	0.771	25– 0	170	84038	494.34
	115	0.739	25– 0	131	63187	482.34
	116	0.740	26–. 0	127	62915	495.39
		(Average) 0.741	(Average) 25– 5	(Total) 531	(Total) 258222	(Average) 486.676
				Standard deviation ±61.233		
Control ♀ non-hibernating ♂non-hibernating	105	0.688	25–19	96	39410	410.52
	106	0.762	25–14	189	87008	460.36
	107	0.737	26– 0	148	66988	452.62
	108	0.752	26– 0	68	32023	470.93
		(Average) 0.735	(Average) 25–19	(Total) 501	(Total) 225429	(Average) 449.551
				Standard deviation ±71.786		

to the following reasons: the bivoltine worms were reared in the autumn under a more favorable environment than the univoltine ones reared at the same time under unfavorable conditions. Accordingly, it might be considered that the experiment shown in table 5 made it appear that the greater vigor of the crossing was caused by the vigor of the bivoltine spermatozoa which were much stronger under a favorable condition than those of the univoltine. If this were true, such spermatozoa influence ought to be also recognized in the experimented worms of table 6. But, on the contrary, we noted that the worms crossed by fertilization with univoltine spermatozoa which became weaker under unfavorable conditions than the bivoltine, nevertheless showed more vigor than the original bivoltine worms. Consequently, it seems more reasonable to consider the vigor of worms as the

result of the surrounding conditions of the embryos, that is, of the ooplasm and nutrients of egg-cells, rather than as proceeding from the direct influence of spermatozoa. When the univoltine worms are inbred, the spermatozoon which entered into the egg-cell is surrounded by its own univoltine ooplasm and nutrients, likewise the bivoltine spermatozoon by its own bivoltine surroundings. On the other hand, in the cross-breeding between these 2 variants, such a spermatozoon is to be controlled by the ooplasm and nutrients which were not in their own normal surroundings. In other words, the former case of inbreeding showed that the nuclei and cytoplasm were not at all modified by any external influence, but in the latter case of the crossing of 2 variants, only the somatic cells of the embryo were modified by the cytoplasm of spermatozoon, and the embryo was physiologically influenced by the blood of the foster mother which, in turn, was changed by the temperature. Consequently, such an embryo would develop in ooplasm and nutrients, forming an abnormal environment for that embryo.

Judging from the results above obtained by experiments on insects, I felt confident that some influence of the cytoplasm of the embryo sac upon the embryos could also be seen in plants. At the suggestion of the author, similar experiments on plants have been carried out by RYI at the GOVERNMENT AGRICULTURAL EXPERIMENT STATION OF CHOSEN. These results will be briefly given in this paper with a view to extending to plants my conclusions here stated. Two groups of Eckendorfer (a pure breed of barley), one of which was obtained by sowing in the autumn and the other in the early spring, were crossed reciprocally at flowering time in the late spring. Then these two crossings were sown at the same time in the autumn with the original self-fertilized. The results obtained in the summer of 1927 were as follows:

TABLE 7

Results of crossings of 2 groups produced from Eckendorfer barley under different environments.

EXPERIMENT AND CONTROL	NUMBER OF STOCKS EXAMINED	DATE OF HEADING	NUMBER OF TILLER	LENGTH			WEIGHT OF PANICLE GRAMS
				STOCK CM	PANICLE CM	AWN CM	
Self-fertilized	108	V-28.6	6.7	93.03	8.79	12.73	16.13
Crossed in different stocks	25	V-28.4	9.6	95.15	9.70	12.73	23.63
♀Sowed in spring×							
♂Sowed in autumn	10	V-28.6	10.0	92.73	10.00	12.42	26.63
♀Sowed in autumn×							
♂Sowed in spring	7	V-28.8	12.1	95.15	9.70	12.73	23.63

As shown in table 7, the crossings between 2 groups produced from a pure barley by sowing it under different conditions showed distinctly higher vigor than the original line self-fertilized. It is most important and interesting to note that a tendency similar to that shown in the previous experiments was also proved in these plants. Moreover, DARWIN (1876) had already observed that cross-fertilization between different flowers in the same individual was better than self-fertilization, and lately YASUDA (1927) proved that in fertility of *Petunia violacea* the greater the difference of physiological conditions of the flowers the better. These observations agree with the results of the experiments carried out by myself and described here.

GENERAL CONSIDERATION

Influence of the ooplasm and nutrients upon the embryo

Whether some conditions of the parents do exert any influence upon the offspring or not is so very important for practical sericulture that it has been repeatedly discussed by many interested authors. The results of the experiments carried out by myself and described here leave no doubt of the existence of such an influence. It is, however, to be expected that the results differ in accordance with the degree of environmental stimuli exerted upon the parents as well as with the difference in environment during the rearing of the offspring. When the conditions in which the worms are reared in the next generation are favorable for growth, it might happen that the influence of the preceding generation might not appear in the least on the worms of the subsequent generation. On the contrary, when the rearing conditions are unfavorable, even a low degree of influence exerted on the preceding generation might appear in the succeeding generation.

But how such a phenomenon occurs is not yet clear. Judging from the results here stated, I presume that it is principally due to the action of the ooplasm and nutrients of the egg-cells which are affected by the blood of the foster mother; this is the case with the very large egg-cells as compared with the spermatozoa of silkworms. As above stated, since hibernating or non-hibernating eggs could be produced by a change in the blood of the foster mother from a bivoltine breed, it is quite probable that, by the alteration of environment, we could also change the blood of the mother, as well as in the case of the alternation of voltinism. If this is assumed, then it could naturally be considered that the embyros must receive some influence through the ooplasm and nutrients of eggs which are controlled by the blood, though the spermatozoa, in some instances, might to some

extent be effective as recognized by the influence of alcohol upon the spermatozoa (STOCKARD 1913, STOCKARD and PAPANICOLAOU 1918). The results of first experiments carried out by ovarian transplantation between the different breeds showed obviously that the embryos were influenced by the ooplasm and nutrients produced by the different blood of the host.

The maintenance of vigor by the worms after they had passed through the embryonal stages, that is, after they had passed out of the environmental influence of the host, could be considered as due to "after-reaction" of the stimulus on the egg-cells.

Vigor resulting from hybridization

The high vigor of the F_1 hybrid and the lowering of this in the F_2 have been observed by many biologists (KOELREUTER, DARWIN, WEISMANN, CASTLE and others) and the cause of it is attributed to the dominant allelomorphs of the forms crossed (CASTLE, SHULL, EAST, KEEBLE, PELLEW, JONES and others). I do not oppose this opinion, but I can not quite agree that the source of the vigor of the F_1 hybrids is found exclusively in the reaction of these genetic factors. If we should try to explain the results of the foregoing experiments by diagrammatic figures and compare them with the F_1 hybrid, the results would be as shown on page 201.

Figure 1 indicates a case of normal inbreeding representing the crossing of a pure breed in which the nuclei a and cytoplasm A of egg-cell and spermatozoon a are not at all modified by any external influence, the embyro aa thus developed typically. Figure 2 indicates a case of ovarian transplantation representing the ooplasm and nutrients A in which the embryo bb is developed, thus giving a quite different environment to the embryo. Figure 3 shows the crossing between 2 variants produced by environmental changes. In this case, the crossing is made between the univoltine and bivoltine variants produced from one and the same bivoltine breed. The symbol a′ indicates a spermatozoon, of which the nucleus is not influenced by the external conditions, but of which the cytoplasm is quite modified by the temperature. Accordingly, such a spermatozoon is naturally adapted to the ooplasm and nutrients A′ of its own normal surroundings. But it must, in this case, fertilize an egg-cell which has a different condition of ooplasm and nutrients A. It may be said in this connection that in fertilization of the germ cells of animals, a part or most of the tail (portion of cytoplasm) of the spermatozoon enters into the egg-cell along with some mitochondria, as already reported by SOBOTTA, MEVES, VAN DER STRICHT, KOSTANECKI, WIERZEJSKI and others, so it leaves no room for doubt that in insects the embryo is influenced by the cytoplasm of the spermatozoon

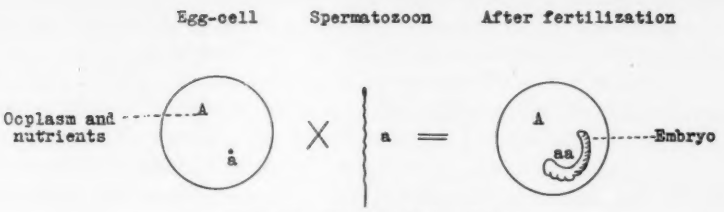

FIGURE 1.—The case of inbreeding.

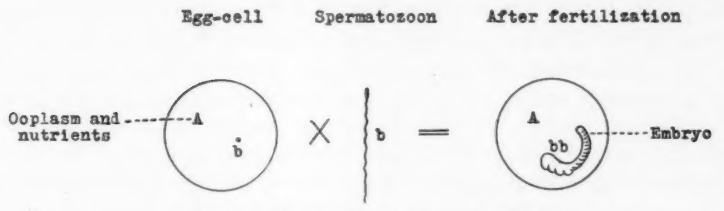

FIGURE 2.—The case of ovarian transplantation.

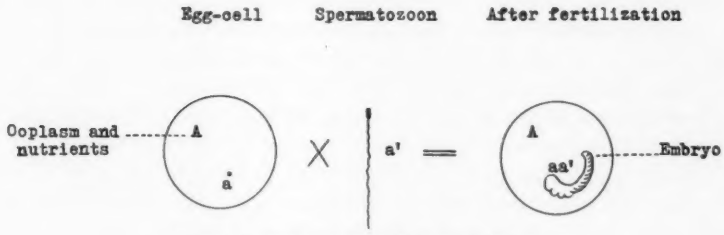

FIGURE 3.—The case of crossing two variants.

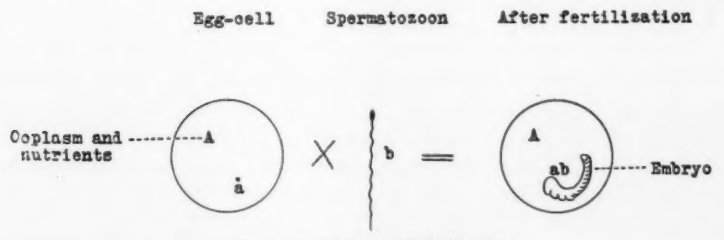

FIGURE 4.—The case of hybridization.

which is affected by its environment. Thus, figure 3 indicates that only the somatic cells of the embryo are modified by the cytoplasm of the spermatozoon, but that its genetic factors are not influenced. Accordingly, such an embryo aa′ must develop in the environment A which is different from that for which the embryo aa′ is adapted, so that this is rather similar to the case indicated in figure 2 (ovarian transplantation), in spite of its resemblance to the case of figure 1 (inbreeding). Lastly, figure 4 represents the usual case of the F_1 hybrid, that is, the embryo ab produced from a heterozygote developing in the ooplasm and nutrients of A. This case rather resembles the cases indicated in figures 2 and 3. If we, therefore, recognize the influence of ooplasm and nutrients upon the embryo as shown in figures 2 and 3, we must also recognize a similar tendency in the case of the F_1 hybrid. Moreover, we can consider that the influence of the hybrid vigor generated by the ooplasm and nutrients is clearly seen in the F_1 generation as shown in table 4, but may scarcely extend to the F_2 hybrid, though we have a rare instance where it may be in a less degree extended to the succeeding generation as we see in table 3. Consequently, it is suggested that the cause of the high vigor of the F_1 hybrid and the lowering of this in the F_2 cannot be looked upon as a result of the reaction of the heterozygotes only, as so many geneticists are inclined to assume.

The maintenance of the influence caused by an environment on the post-embryonal stages of the hybrid as well as on the worms of later generation should also be considered as a case of "after-reaction." Hence, there is nothing against the assumption that the high vigor of hybridization is due, in addition to the reaction of the dominant heterozygotes, to the stimulus exerted upon the developing embryos by the ooplasm and nutrients of the egg-cells. For this reason, strictly speaking, the term "heterosis" should not be limited to cases of hybrid vigor.

SUMMARY

1. In the ovarian transplantation between different breeds of silkworms, the individuals reared from an ovary transplanted into a different breed always showed a shorter duration of larval life, a greater weight and higher reproductivity than those of the control, especially in the case where the operation of transplantation was successfully carried out and the individual into which the ovaries were transplanted was fully developed and was not physiologically injured by bleeding from the slit made when it was operated upon.

2. The worms reared from the transplanted ovaries of a pure breed greatly weakened by successive inbreeding showed more strongly the in-

vigorating influence of the host than did the other pure breed which showed no bad effects of inbreeding.

3. Crosses between univoltine and bivoltine worms produced from a pure bivoltine stock under different environments also showed higher vigor than those of the original breed, as the above experiments indicated. And such phenomena as these in insects have also been observed in the crossing of two groups produced from a pure barley.

4. The question as to whether some conditions of the parents do exert any influence upon the offspring or not depends upon the degree of environmental stimulus exerted upon the parents as well as upon the difference of environment during the rearing of the offspring. And, in silkworms, the egg-cells of which are much larger than the spermatozoa, such an influence upon the offspring is principally due to the after-reaction of the stimulus of the ooplasm and nutrients controlled by the blood of the foster mother though in rare instances it is due to the spermatoza.

5. It is reasonable to suggest that the cause of the high vigor of hybridization is due, not only to the reaction of the dominant allelomorphs, as so many geneticists are inclined to assume, but also to the after-reaction of the stimulus exerted upon the developing embryos by the ooplasm and nutrients of the egg-cells. Accordingly, the term "heterosis," strictly speaking, should not be limited to cases of vigor due to hybridization.

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CROSSING OVER IN THE THIRD CHROMOSOMES OF TRIPLOIDS OF *DROSOPHILA MELANOGASTER**

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INTRODUCTION

Upon *a priori* grounds it has long been assumed that any crossover involves at least two phenomena: (1) a rotation or overlapping of chromosomes or of their derivative strands, and (2) a breaking and reunion at one or more loci of these strands. (For a recent discussion see BELLING 1927.) It is known in Drosophila that the segmental exchange does not occur in males and that it takes place in diploid females at the four-strand stage (BRIDGES 1916, ANDERSON 1925, L. V. MORGAN 1925, STURTEVANT unpublished) and in triploid females at the corresponding six-strand stage (BRIDGES and ANDERSON 1925). Further evidence indicates that the process occurs in early oöcytes before the first maturation division (PLOUGH 1917, 1921; GOWEN 1929). The amount of double crossing over is dependent upon the distance between the loci involved and varies with the region of the chromosome under observation (BRIDGES and MORGAN 1923). Crossing over may be partially or completely inhibited by "crossover reducers" (STURTEVANT 1919, GOWEN 1928, etc.) some of

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which have been shown to be inverted sections of chromosome (STURTEVANT 1926).

A number of recent results indicate a correlation between the effects of various agents and the distance of the region involved from the spindle-fibre attachment. Using young females as contrasted with older females, or subjecting females to temperatures greater or less than about 25°C, or to X-rays, results in a considerable increase in crossing over in the region of spindle-fibre attachment (BRIDGES 1915, 1927; PLOUGH 1917, 1921; MAVOR 1923; MAVOR and SVENSON 1924; MULLER 1925, 1926; STERN 1926). This is undoubtedly true of the V-shaped second and third chromosomes in which the attachment is at the apex of the V, and is probably true also of the rod-shaped X chromosome in which the attachment is terminal and at the right end of the chromosome map. The fourth chromosome has not been tested.

Whatever the mechanical factors at work, it would seem that a substitution of six sets of crossover strands in the triploid for the four sets ordinarily present in the diploid might throw some light upon the process of crossing over. However, besides the mere substitution of a greater number of chromosome strands, triploidy involves changes in the general relationships within the cell, and, in particular, possible changes in the ratio of chromatin to cytoplasm as regards surface and volume. Such changes in themselves might be responsible for the crossover variations found. This question will be considered later.

One of the main difficulties encountered in the analysis of triploid data relates to the possibility of crossing over of sister strands (that is, the two strands resulting from the splitting of a single chromosome). Crossing over is ordinarily measured by the percentage of crossed-over chromosomes detected. But in both diploid and triploid, crossing over between sister strands, if it occurs, will remain undetected. If it is as likely to occur between sister strands as between non-sister strands, then in the diploid in any given region the undetected crossing over would be $\frac{1}{3}$ the actual total crossing over, that is, the ordinary "crossover" values would represent $\frac{2}{3}$ the actual physical crossing over. But in the triploid, since six strands are involved, only $\frac{1}{3}$ the total crossing over would be undetected—in other words, the detected "crossing over" would represent $\frac{5}{6}$ the total crossing over.

A simple correction may, of course, be made for sister-strand crossing over in comparing diploid with triploid, but it rests upon the assumption of free sister-strand interchange. It was expected, as a matter of fact,

that the possibility of such interchange could be investigated by means of data from triploids. Unfortunately, as BRIDGES and ANDERSON (1925) also found, the difference between triploid and diploid shows such great variation in different regions of the chromosome that any effect to which sister-strand interchange may give rise is completely masked. Corrected values will be tabulated whenever it seems advisable. These corrections are superfluous, if, as the recent paper of STURTEVANT (1928) indicates, there is no sister-strand crossing over. He finds that controlled cases of reversion of bar eye to round are always accompanied by detected crossing over. If sister-strand crossing over occurred at all in such crosses, it should have given many more apparent non-crossover reversions than can be explained on the basis of experimental error. On the other hand it is possible that bar reversion represents a special case, and if so there is no way of knowing at present whether or not sister-strand interchange takes place. However, as will be shown later (page 236), the conclusions of the present paper in regard to the differences between crossing over in triploid and diploid remain unaltered, even if sister strands cross over as freely as non-sister strands.

CROSSES IN WHICH THE THREE THIRD CHROMOSOMES WERE MARKED

Technically the study of crossing over in triploids is made difficult by a number of circumstances. Thus, relatively few offspring are obtained, and these offspring are of various types in regard to their chromosome balance. They include approximately: 3.9 percent triploid daughters, 46.8 percent diploid daughters, 6.0 percent diploid sons, 36.9 percent intersexes, 1.3 percent superfemales, and 5.2 percent supermales (see BRIDGES and ANDERSON 1925). The facts that some expected chromosome combinations do not appear at all (for example, individuals diploid except for the presence of three second or third chromosomes) and that the percentages of the types which do appear are very different from expectation, are believed to depend upon viability differences during development.

In the investigations of BRIDGES and ANDERSON on crossing over in the X chromosome advantage was taken of the fact that of the 46.8 percent diploid daughters produced, practically all (that is, 41.1 percent of the total offspring) represent exceptions which receive a Y chromosome from the father, the two X chromosomes coming from the triploid mother. Data on crossing over for this chromosome may then be obtained by genetic tests of the composition of the exceptional daughters.

But the corresponding exceptions for the two larger autosomes do not appear, and this technique is therefore not applicable in the study of chromosome III. The diploid sons and daughters have each received one third chromosome from the triploid mother and one from the diploid father. Crossing over is then measured directly by the relative proportions of the diploid types emerging from the cross.

In the crosses first successfully utilized all three third chromosomes were marked according to the scheme previously adopted by BRIDGES and ANDERSON. A series of mutant loci were chosen, such that very near each locus another favorable mutant gene is known for each of the points chosen; the two genes and the normal locus of the first were introduced into the triploid, one in each of the three chromosomes, and were treated virtually as a series of allelomorphs. Actually one is non-allelomorphic but is so close to the other locus that crossing over between them is relatively negligible. Because the characters which it seemed advisable to use interfere to a certain extent with each other, it was necessary, in covering the chromosome, to make use of two crosses instead of one. These crosses are as follows:

(1)
$$\frac{+ \quad D \quad m_a \quad S_b \quad +}{s_e \quad M_h \quad + \quad + \quad H} \times s_e\ h\ m_a\ c_u\ b_x\ e^s \text{♂}.$$
$$\frac{}{h \quad + \quad c_u \quad b_x \quad e^s} \text{♀}$$

(2)
$$\frac{+ \quad H \quad +}{S_b \quad + \quad M} \times b_x\ e^s\ c_a \text{♂}.$$
$$\frac{}{b_x \quad e^s \quad c_a} \text{♀}$$

The symbols stand for the following third chromosome mutants, the genes having the positions indicated on the standard diploid map: s_e, sepia at 26.0 and h, hairy one-half unit to the right of sepia; M_h, minute-h at 40.1 and D, dichaete at 40.4; m_a, maroon at 49.7 and c_u, curled at 50.0; S_b, stubble at 58.2 and b_x, bithorax at 58.7; H, hairless at 69.5 and e^s, sooty at 70.7; c_a, claret at 100.7 and M, minute at 101.0.

Of the seven special stocks needed for the work, only hairless was already in existence; it was necessary therefore to synthesize the others. Each stock was derived from a single chromosome which was then (excepting $h\ c_u\ b_x\ e^s$ and $s_e\ h\ m_a\ c_u\ b_x\ e^s$) kept intact by being carried over the

$lC_L\ C_R$ combination which prevents crossing over. Each was then inbred for several generations before being used.

The appearance of the triploid with a single dose of the mutant genes involved is of some interest. As would be expected, the recessives do not become manifest. A dominant, on the other hand, may or may not have an observable effect. Thus minute and minute-h do not appear in single dose; hairless however has a distinct effect but is less extreme than in the diploid. And, finally, the manifestation of dichaete and stubble does not differ appreciably in triploid and diploid.

There are certain pronounced difficulties connected with the use of the stocks. In the first place it is impossible to distinguish between sepia and the sepia-maroon double recessive. This makes it necessary either to introduce a third eye color which behaves as a sensitizer and thus aids in the separation of the various eye-color types, or to test genetically all sepia-appearing diploid offspring (a third of all the diploids) for the presence of maroon. The genetic test was used until late in the work, when it was discovered that vermilion (a sex-linked recessive) behaves as a sensitizer. At this stage all the stocks were accordingly made homozygous for vermilion. But even in the presence of vermilion, the vermilion-sepia eye (a brassy orange color) and the vermilion-sepia-maroon eye (lemon yellow) become indistinguishable after the first half-day. It is necessary then to classify the flies before they are more than about ten hours old.

In the second place most of the dominants used are lethal in double dose in triploids and intersexes. This is true of minute, minute-h and dichaete; double hairless occasionally survives but is very extreme in appearance. Although double stubble was not detected in these experiments, it frequently survives, according to the unpublished data of SCHULTZ. In these crosses, due to the lethal effects, it is not possible to determine the position of the spindle-fibre attachment, for this determination depends upon the relative numbers of equational exceptions at the various loci (see page 245). There are further difficulties, in particular those connected with obtaining triploids of the proper composition to test, but these need not be discussed here.

The diploid controls include the following crosses from the same stocks used for the triploid crosses:

(1) $$\frac{s_e\ \ M_h\ \ +\ \ +\ \ H}{h\ \ +\ \ c_u\ \ b_z\ \ e^s}\ \text{♀} \times s_e\ h\ m_a\ c_u\ b_z\ e^s\ \text{♂}.$$

(2) $\frac{+ \quad D \quad m_a \quad S_b \quad +}{h \quad + \quad c_a \quad b_z \quad e^s} \text{♀} \times s_e\, h\, m_a\, c_a\, b_z\, e^s \text{♂}.$

(3) $\frac{+ \quad H \quad +}{b_z \quad e^s \quad c_a} \text{♀} \times b_z\, e^s\, c_a \text{♂}.$

(4) $\frac{+ \quad H \quad +}{S_b \quad + \quad M} \text{♀} \times b_z\, e^s\, c_a \text{♂}.$

The actual procedure was to isolate a diploid or triploid virgin of the required composition and to put her into a small vial with five males from the proper tester stock. These six flies were allowed to remain in the vial one day, after which they were transferred to the usual half-pint culture bottle provided with the cornmeal-molasses-agar medium. After five days in the first culture bottle they were transferred to a second new culture bottle and remained there five days. In the early stages of the work a transfer was then made to a third culture bottle, but it soon became obvious that triploid females give practically no offspring in these third cultures; and all females were thereafter discarded after the first eleven days of their life. These transfers were, of course, made in order to detect any gross variations with age. A more delicate test of the effect of age did not seem advisable.

The data for these triploid and diploid crosses appear in tables 1, 2, 3, and 4. In these tables the numerals "I" and "II" refer to the first and second cultures described above, that is "I" includes flies hatched in the first cultures from eggs laid on the second to the sixth days inclusive of the mother's life, and "II" includes flies from the second cultures from eggs laid on the seventh to the eleventh days.

The data from triploid mothers are given in full in tables 1 and 3. Since the control data (tables 2 and 4) are ordinary diploid data it was not considered necessary to present them in similar space-consuming form. The short laying period and the care taken of the culture bottles insured a reasonable equality of contrary classes; no viability differences appeared which were great enough to necessitate corrections.

In the data from triploids of the type $a_1a_2a_3 \cdots / b_1b_2b_3 \cdots / c_1c_2c_3 \cdots$ two types of double crossovers are to be distinguished. The first are the so-called "recurrent" doubles in which the same two chromosomes are involved in the two succeeding crossings over (for example $a_1b_2a_3$); the

TABLE 1

Offspring of the cross $\dfrac{+\ D\ m_a\ S_b\ +}{s_e\ M_h\ +\ +\ H}$ ♀ × $s_e\ h\ m_a\ c_u\ b_z\ e^s$ ♂. *R indicates recurrent double crossovers; P, progressive double crossovers.*

	I; 21 CULTURES	II; 18 CULTURES
Triploids	29	19
♀ Intersexes	186	129
♂ Intersexes	19	19
Superfemales	..	1
Supermales	..	2

Diploids

Type	Classes	♀	♂	Total	♀	♂	Total
0	$D\ m_a\ S_b$	103	15	340	68	18	225
	$s_e\ M_h\ H$	95	14		67	5	
	$h\ c_u\ b_z\ e^s$	93	20		62	5	
1	$s_e\ D\ m_a\ S_b$	9	..	47	7	..	37
	$M_h\ H$	7	..		5	..	
	$h\ D\ m_a\ S_b$	8	3		7	1	
	$c_u\ b_z\ e^s$	6	..		3	..	
	$s_e\ c_u\ b_z\ e^s$	9	..		6	2	
	$h\ M_h\ H$	5	..		6	..	
2	$D\ H$	12	1	110	8	2	61
	$s_e\ M_h\ m_a\ S_b$	9	3		4	..	
	$D\ c_u\ b_z\ e^s$	15	1		15	1	
	$h\ m_a\ S_b$	18	4		9	1	
	$h\ H$	24	..		12	..	
	$s_e\ M_h\ c_u\ b_z\ e^s$	22	1		7	2	

TABLE 1 (*continued*)

Diploids			I; 21 CULTURES			II; 18 CULTURES		
Type		Classes	♀	♂	Total	♀	♂	Total
3		*D* m_a *H*	8	1	40	7	1	29
		s_c M_h S_b	5	2		4	1	
		D m_a b_x e^a	7	2		2	..	
		h c_u S_b	6	1		6	1	
		h c_u *H*	4	1		3	..	
		s_e M_h b_x e^a	2	1		2	2	
4		*D* m_a S_b *H*	2	..	29	5	..	17
		s_e M_h	4	1		1	1	
		D m_a S_b e^a	7	1		1	..	
		h c_u b_x	2	..		4	..	
		h c_u b_x *H*	7	1		1	..	
		s_e M_h e^a	4	..		3	1	
1,2	R	s_e *D H*	2	..	16	1	..	5
		M_h m_a S_b	4	..		..	..	
		m_a S_b	2	..		..	..	
		h D c_u b_x e^a	..	..		..	..	
		s_e *H*	..	..		..	..	
		h M_h c_u b_x e^a	..	..		..	..	
	P	*H*	1	..		1	..	
		M_h c_u b_x e^a	2	..		..	..	
		s_e m_a S_b	1	..		1	..	
		s_e *D* c_u b_x e^a	..	..		..	..	
		h D H	3	..		1	..	
		h M_h m_a S_b	1	..		..	1	

TABLE 1 (*continued*)

Diploids			I; 21 CULTURES			II; 18 CULTURES		
Type	Classes		♀	♂	Total	♀	♂	Total
1,3	R	s_e D m_a H	1	..	7	..	..	4
		M_h S_b	1	..		..	..	
		h D m_a b_x e^a	..	..		..	..	
		c_u S_b	1	..		..	..	
		s_e c_u H	1	..		..	..	
		h M_h b_x e^a	..	..		..	..	
	P	c_u H	..	..		1	..	
		M_h b_x e^a	1	..		1	..	
		s_e D m_a b_x e^a	..	..		..	..	
		s_e c_u S_b	..	..		..	..	
		h D m_a H	1	..		..	1	
		h M_h S_b	1	..		1	..	
1,4	R	s_e D m_a S H	..	..	4	1	..	3
		M_h	..	..		..	..	
		c_u b_x	..	..		..	..	
		h D m_a S_b e^a	..	..		..	..	
		s_e c_u b_x H	..	..		..	..	
		h M_h e^a	..	..		..	..	
	P	c_u b_x H	1	..		..	..	
		M_h e^a	1	..		1	..	
		s_e D m_a S_b e^a	..	..		1	..	
		s_e c_u b_x	..	..		..	..	
		h D m_a S_b H	1	..		..	..	
		h M_h	1	..		..	..	

TABLE 1 (*continued*)

Diploids			I; 21 cultures			II; 18 cultures		
Type	Class		♀	♂	Total	♀	♂	Total
2,3	R	*D* S_b	3	1	22	1	..	5
		s_e M_h m_a *H*	1	..		..	..	
		D c_u S_b	2	..		..	..	
		h m_a b_x e^a	2	..		1	..	
		h b_x e^a	2	..		..	..	
		s_e M_h c_u *H*	1	..		..	..	
	P	*D* b_x e^a	3	..		..	..	
		D c_u *H*	..	1		..	..	
		s_e M_h m_a b_x e^a	..	..		1	..	
		s_e M_h c_u S_b	1	..		1	..	
		h m_a *H*	1	..		..	..	
		h S_b	3	1		1	..	
2,4	R	*D*	1	..	10	1	..	2
		s_e M_h m_a S_b *H*	..	..		..	..	
		D c_u b_x	..	..		..	..	
		h m_a S_b e^a	2	..		1	..	
		h e^a	1	..		..	..	
		s_e M_h c_u b_x *H*	..	..		..	..	
	P	*D* e^a	..	..		..	..	
		D c_u b_x *H*	2	..		..	..	
		s_e M_h m_a S_b e^a	2	..		..	..	
		s_e M_h c_u b_x	2	..		..	..	
		h m_a S_b *H*	..	..		..	..	
		h	..	..		..	..	

TABLE 1 (*continued*)

Diploids			I; 21 CULTURES			II; 18 CULTURES		
Type	Class		♀	♂	Total	♀	♂	Total
3,4	R	*D* m_a	..	..	5	..	..	1
		s_e M_h S_b *H*	..	..		..	..	
		D m_a b_x	..	..		..	..	
		h c_u S_b e^s	3	..		..	..	
		h c_u e^s	..	..		..	..	
		s_e M_h b_x *H*	..	..		..	..	
	P	*D* m_a e^s	..	..		..	..	
		D m_a b_x *H*	1	..		..	..	
		s_e M_h S_b e^s	..	..		..	..	
		s_e M_h b_x	..	..		..	1	
		h c_u S_b *H*	..	..		..	..	
		h c_u	1	..		..	..	
1,2,3		*h* M_h c_u S_b	..	..	..	1	..	1
1,2,4		s_e *D*	..	..	..	..	1	1
1,2,4		*h* M_h m_a S_b e^s	..	..	..	1	..	1
1,3,4		*h D* m_a	1	..	1	..	..	..
1		s_e *h* c_u b_x e^s	1	..	1	1	..	1
1		s_e M_h *D* m_a S_b	1	..	1	..	..	..
3		*D* m_a S_b b_x e^s	..	..	..	1	..	1
4		s_e M_h *H* e^s	1	..	1	..	..	..
1,2	P	s_e *D* m_a c_u b_x e^s	1	..	1	..	..	..
1,4	P	e^s	..	..	..	1	..	1
		Total	559	76	635	348	47	395

TABLE 2

Offspring from control diploid mothers of the composition indicated; the fathers were s_e h m_a c_u b_z e^s.

MOTHER	TYPE	0	1	2	3	4	1,2	1,3	1,4	2,3	2,4	3,4	1,2,3	1,2,4	1,3,4	2,3,4	TOTAL
$+ D\ m_a\ S_b +$ / $h + c_u\ b_z\ e^s$	I; 15 cultures	1654	258	127	132	151	10	14	33	13	21	21	3	..	..	..	2437
	II; 14 cultures	1849	240	77	119	162	6	7	29	6	15	9	..	1	2	..	2522
$s_e\ M_h + +$ H / $h + c_u\ b_z\ e^s$	I; 13 cultures	1525	289	133	132	205	5	29	33	6	14	4	..	..	1	2	2378
	II; 12 cultures	1758	267	102	105	212	1	23	29	11	11	6	1	1	..	3	2530
Total	I; 28 cultures	3179	547	260	164	356	15	43	66	19	35	25	3	..	1	2	4815
	II; 26 cultures	3607	507	179	224	374	7	30	58	17	26	15	1	2	2	3	5052

TABLE 3

Offspring of the cross $\frac{+\ H\ +}{S_b + M}$ ♀ × $b_z\ e^a\ c_a$ ♂.

$b_z\ e^a\ c_a$

	I; 28 CULTURES	II; 27 CULTURES
Triploids	80	75
♀ Intersexes	353	319
♂ Intersexes	84	107
Superfemales	..	..
Supermales	4	8

Diploids

Type	Class	♀	♂	Total	♀	♂	Total
0	*H*	215	40	649	189	38	574
	$S_b\ M$	158	28		141	32	
	$b_z\ e^a\ c_a$	180	28		152	22	
4	$S_b\ H$	9	2	69	8	2	58
	M	13	2		11	1	
	$b_z\ H$	7	..		9	..	
	$e^a\ c_a$	15	2		10	2	
	$S_b\ e^a\ c_a$	15	..		9	2	
	$b_z\ M$	3	1		4	..	
5	S_b	27	5	173	24	5	151
	$H\ M$	31	5		16	2	
	$H\ c_a$	24	7		31	4	
	$b_z\ e^a$	28	6		21	4	
	$S_b\ c_a$	27	3		29	3	
	$b_z\ e^a\ M$	8	2		11	1	

TABLE 3 (*continued*)

Diploids			I; 28 CULTURES			II; 27 CULTURES		
Type		Class	♀	♂	Total	♀	♂	Total
4,5	R	+	4	..	17	2	..	13
		S_b *H M*	1	..		1	..	
		b_x *H* c_a	..	..		1	..	
		e^s	1	..		1	..	
		S_b e^s *M*	..	..		1	..	
		b_x c_a	3	1		1	..	
	P	c_a	..	1		..	..	
		S_b *H* c_a	..	1		1	..	
		b_x *H M*	1	..		1	..	
		b_x	1	..		1	..	
		S_b e^s	..	..		2	..	
		e^s *M*	2	1		..	1	
4		*H* e^s c_a	2	..	2	..	..	..
4,5	R	S_b b_x e^s *M*	..	..	..	1	..	1
		Total	775	135	910	678	119	797

second are the "progressive" doubles in which all three chromosomes are involved two at a time (for example $c_1a_2b_3$). These are indicated in tables 1 and 3 by "R" and "P" respectively.

It will be seen that there is a certain expected amount of crossing over between the two mutant genes treated as markers of a given point (for example between *H* and e^s). In order to incorporate these crossovers the convention was followed that the first region includes the distance from sepia to dichaete; all other regions extend from the second gene of a given pair to the second gene of the succeeding pair. It is true that a very small amount of crossing over will remain undetected due to the fact that one of the markers of a given point is the normal allelomorph of one of the mutants, but this is insignificant.

TABLE 4

Offspring from control diploid mothers of the composition indicated; the fathers were $b_x\ e^s\ c_a$.

MOTHER	TYPE	0	4	5	4.5	TOTAL
$+\ H\ +$	I; 13 cultures	1684	270	843	64	2861
$b_x\ e^s\ c_a$	II; 12 cultures	1206	180	585	25	1996
$+\ H\ +$	I; 23 cultures	2455	433	1377	57	4322
$S_b + M$	II; 17 cultures	1640	237	766	23	2666
Total	I; 36 cultures	4139	703	2220	121	7183
	II; 29 cultures	2846	417	1351	48	4662

CROSSES IN WHICH ONE THIRD CHROMOSOME WAS MARKED

When the triploid data presented in the preceding section were analyzed the results were so unexpected that it was thought well to repeat the work with entirely different stocks. Accordingly the following triploid cross and its diploid control cross were used:

1) $\dfrac{\dfrac{r_u\ h\ t_h\ s_t\ c_u\ s_r\ e^s\ c_a}{+}}{+}$ ♀ × $r_u\ h\ t_h\ s_t\ c_u\ s_r\ e^s\ c_a$ ♂.

(2) $$\frac{r_u\ h\ t_h\ s_t\ c_u\ s_r\ e^s\ c_a}{+}\ \text{♀} \times r_u\ h\ t_h\ s_t\ c_u\ s_r\ e^s\ c_a\ \text{♂}.$$

The technique employed was quite similar to that described for the previous crosses except that ten males instead of five were used for every female in order to insure proper fertilization. The symbols represent the following mutant genes which have the loci indicated on the standard diploid map: r_u, roughoid at 0.0; h, hairy at 26.5; t_h, thread at 42.2; s_t, scarlet at 44.0; c_u, curled at 50.0; s_r, stripe at 62.0; e^s, sooty at 70.7; c_a, claret at 100.7.

In utilizing the data of cross (1) a correction must be made for undetected crossing over between the two similar wild-type chromosomes. If we consider any given region, for example region (4) between scarlet and curled, the amount of crossing over between the marked chromosome and the two wild-type chromosomes is known. But the amount of crossing over in region (4) between the two wild-type chromosomes is not known. If we assume this latter to be half the former (and, as will be seen, the results justify this assumption), then the crossing over for all three chromosomes will be $1\frac{1}{2}$ times the detected crossing over. (This correction, it should be noted, has nothing to do with a correction for sister-strand crossing over.)

A more explicit demonstration of this relationship is quite simple, but tedious. A brief resumé follows.

We may indicate the marked chromosome of the triploid by $a_1a_2a_3a_4a_5a_6a_7a_8$; and the wild-type chromosomes of this triploid by $b_1b_2b_3b_4b_5b_6b_7b_8$ and $b_1'b_2'b_3'b_4'b_5'b_6'b_7'b_8'$. We have then individuals of the following composition and appearance which are represented in the proportions indicated. That the various classes of a given type are equal for the first experiment is shown at once by a glance at table 1; it is assumed that the chromosomes of the present experiment behave similarly.

Crossover region	*Composition*	*Appearance*	*Proportion*	
0	$a_1a_2a_3a_4a_5a_6a_7a_8$	$a_1a_2a_3a_4a_5a_6a_7a_8$	s	3s
	$b_1b_2b_3b_4b_5b_6b_7b_8$	$b_1b_2b_3b_4b_5b_6b_7b_8$	*s*	
	$b_1'b_2'b_3'b_4'b_5'b_6'b_7'b_8'$	$b_1b_2b_3b_4b_5b_6b_7b_8$	s	

	$a_1b_2b_3b_4b_5b_6b_7b_8$	$a_1b_2b_3b_4b_5b_6b_7b_8$	t	
	$b_1a_2a_3a_4a_5a_6a_7a_8$	$b_1a_2a_3a_4a_5a_6a_7a_8$	t	
	$a_1b_2'b_3'b_4'b_5'b_6'b_7'b_8'$	$a_1b_2b_3b_4b_5b_6b_7b_8$	t	
1	$b_1'a_2a_3a_4a_5a_6a_7a_8$	$b_1a_2a_3a_4a_5a_6a_7a_8$	t	6t
	$b_1b_2'b_3'b_4'b_5'b_6'b_7'b_8'$	$b_1b_2b_3b_4b_5b_6b_7b_8$	t	
	$b_1'b_2b_3b_4b_5b_6b_7b_8$	$b_1b_2b_3b_4b_5b_6b_7b_8$	t	
2	6 classes	6 classes	6u	
3	"	"	6v	
4	"	"	6w	
5	"	"	6x	
6	"	"	6y	
7	"	"	6z	
1,2	12 classes	12 classes	$12c_1$	
1,3	"	"	$12c_2$	
.	.	.	.	
.	.	.	.	
.	.	.	.	
.	.	.	.	
5,7	12 classes	12 classes	$12c_{20}$	
6,7	"	"	$12c_{21}$	
1,2,3	24 classes	24 classes	$24d_1$	
1,2,4	"	"	$24d_2$	
.	.	.	.	
.	.	.	.	
4,6,7	24 classes	24 classes	$24d_{34}$	
5,6,7	"	"	$24d_{35}$	
1,2,3,4	48 classes	48 classes	$48e_1$	
1,2,3,5	"	"	$48e_2$	
.	.	.	.	
.	.	.	.	
3,5,6,7	48 classes	48 classes	$48e_{34}$	
4,5,6,7	"	"	$48e_{35}$	

We need not consider the possibility of further multiple crossovers since they do not occur. Their presence would not alter the expressions below except by the addition of terms.

Let us now consider the total amount of crossing over between any two

given loci, for example between loci 4 and 5. By collecting terms we obtain the following expressions:

The total crossing over $= 6w+12(c_3+c_8+c_{12}+c_{16}+c_{17}+c_{18})+24(d_2+d_6+d_{10}+d_{11}+d_{12}+d_{16}+d_{20}+d_{21}+d_{22}+d_{26}+d_{27}+d_{28}+d_{32}+d_{33}+d_{34})+48(e_1+e_5+e_6+e_7+e_{11}+e_{12}+e_{13}+e_{17}+e_{18}+e_{19}+e_{21}+e_{22}+e_{23}+e_{27}+e_{28}+e_{29}+e_{31}+e_{32}+e_{33}+e_{35})$.

The detectable crossing over $=4w+8(c_3+c_8+c_{12}+c_{16}+c_{17}+c_{18})+16(d_2+d_6+d_{10}+d_{11}+d_{12}+d_{16}+d_{20}+d_{21}+d_{22}+d_{26}+d_{27}+d_{28}+d_{32}+d_{33}+d_{34})+32(e_1+e_5+e_6+e_7+e_{11}+e_{12}+e_{13}+e_{17}+e_{18}+e_{19}+e_{21}+e_{22}+e_{23}+e_{27}+e_{28}+e_{29}+e_{31}+e_{32}+e_{33}+e_{35})$.

That is, the crossing over between loci 4 and 5 is $1\frac{1}{2}$ times the detected crossing over. It may be similarly shown that no matter which two loci are chosen, the crossing over between them is $1\frac{1}{2}$ times the detected crossing over.

Up to this point the discussion has dealt with crossing over and with "crossover values," that is, with the number of crossovers that fall between two given loci for each hundred gametes. But this value cannot be obtained directly, for double crossing over may occur between two given loci without changing the combination of the genes in the loci. By experiments in which other loci between the two given loci are marked, the frequency of these doubles can be determined. These frequencies thus provide corrections by which the percentages of recombination of the genes of the given loci, which is the relation that is directly observed in an experiment, can be transformed into the desired crossover values. For the short intervals dealt with in this paper the amount of this correction is small (see page 241), and the recombination percents are used as indices of the conditions with respect to crossing over.

Table 5 gives the data in complete form for both triploid and diploid crosses in which one chromosome is marked. It should be noticed that the contrary classes of the diploid crosses are on the whole quite well balanced for viability. Indeed, for the type of cross made, the viability relations of the classes involved are unexpectedly good.

The "contrary" classes of the triploid crosses are not, of course, expected to be equal. The wild-type class, for example, includes not only the wild-type non-crossovers, but all the crossovers involving the two wild-type chromosomes alone. But that the viability in the classes of the triploid crosses is good is shown by the strict correspondence between the crossover

values obtained from these triploids and the values obtained from the triploids in which all three chromosomes were marked (see pages 234, 236).

THE RECOMBINATION VALUES

Table 6 presents the recombination values for the first experiment calculated from the data of tables 1, 2, 3, and 4. These figures represent the percentages of *detected* crossed-over chromosomes which emerge. They are not corrected for a possible sister-strand crossing over. The differences between the recombination values for triploid and for diploid, as well as the quotients of these values, are listed separately. It is clear that at the end of the chromosome included in this experiment (the right end) recombination for the triploid is almost half that for the diploid; and that there is a continuous increase in the relative amount of triploid crossing over as we pass from either end of the chromosome to the center. Thus, in the two regions from s_e, h to M_h, D and from S_b, b_z to H, e^s, triploid recombination is only slightly less than diploid recombination. But for the center of the chromosome, triploid recombination is over $3\frac{1}{2}$ times as great as diploid recombination. These relationships hold for both the first and the second sets of cultures. It should be noted that the quotients have relatively low probable errors.

TABLE 5

Offspring of triploids of the composition (r_u h t_h s_t c_u s_r e^s c_a/+/+) and of control diploids of the composition (r_u h t_h s_t c_u s_r e^s c_a/+); the fathers were r_u h t_h S_t c_u s_r e^s c_a. For the offspring from triploids the "types" indicate the apparent regions affected (see text).

	TRIPLOID		DIPLOID	
	I; 29 cultures	II; 25 cultures	I; 10 cultures	II; 10 cultures
Triploids	91	73	..	..
♀ Intersexes	339	251	..	..
♂ Intersexes	202	142	..	..
Superfemales	..	..	..	..
Supermales	5	3	..	..

Diploids

"Type"	Class	♀	♂	Total	♀	♂	Total	♀	♂	Total	♀	♂	Total
0	r_u h t_h s_t c_u s_r e^s c_a	64	7	462	55	6	309	177	162	754	113	115	617
	+	338	53		208	40		218	197		193	196	
1	r_u	27	6	58	26	3	43	96	68	293	67	80	234
	h t_h s_t c_u s_r e^s c_a	22	3		12	2		72	57		50	37	
2	r_u h	32	3	50	18	2	29	60	58	125	61	43	188
	t_h s_t c_u s_r e^s c_a	12	3		8	1		50	57		39	45	
3	r_u h t_h	..	..	1	..	..	1	2	..	..	..	..	2
	s_t c_u s_r e^s c_a	1	..		1	..		3	3	8	1	1	

TABLE 5 (*continued*)

4	$r_u\ h\ l_h\ s_l$	30	4	69	21	4	45	19	10	60	11	7	30
	$c_u\ s_r\ e^s\ c_a$	30	5		19	1		12	19		7	5	
5	$r_u\ h\ l_h\ s_l\ c_u$	19	1	57	14	1	35	31	33	168	20	15	91
	$s_r\ e^s\ c_a$	32	5		19	1		51	53		29	27	
6	$r_u\ h\ l_h\ s_l\ c_u\ s_r$	5	..	16	2	1	14	21	11	116	23	20	111
	$e^s\ c_a$	10	1		9	2		42	42		36	32	
7	$r_u\ h\ l_h\ s_l\ c_u\ s_r\ e^s$	12	1	50	8	..	29	103	85	419	79	65	381
	c_a	33	4		19	2		119	112		123	114	
1,2	$r_u\ l_h\ s_l\ c_u\ s_r\ e^s\ c_a$	1	..	8	3	..	5	1	..	13	..	..	5
	h	4	3		2	..		7	5		3	2	
1,3	$r_u\ s_l\ c_u\ s_r\ e^s\ c_a$	..	..	..	..	..	..	..	..	..	..	..	..
	$h\ l_h$	..	..		..	..		..	..		..	..	
1,4	$r_u\ c_u\ s_r\ e^s\ c_a$	4	..	18	..	..	9	3	3	12	4	2	11
	$h\ l_h\ s_l$	10	4		8	1		4	2		3	2	
1,5	$r_u\ s_r\ e^s\ c_a$	..	1	3	1	..	2	25	19	68	11	8	40
	$h\ l_h\ s_l\ c_u$	1	1		1	..		10	14		13	8	

TABLE 5 (*continued*)

Diploids		I; 29 CULTURES			II; 25 CULTURES			I; 10 CULTURES			II; 10 CULTURES		
"Type"	Class	♀	♂	Total	♀	♂	Total	♀	♂	Total	♀	♂	Total
1,6	$r_u\ e^a\ c_a$	1	1	5	2	..	2	16	13	42	14	21	49
	$h\ t_h\ s_t\ c_u\ s_r$	2	1		..	..		6	7		9	5	
1,7	$r_u\ c_a$	3	..	11	2	..	6	60	44	195	47	42	140
	$h\ t_h\ s_t\ c_u\ s_r\ e^a$	7	1		4	..		53	38		20	31	
2,3	$r_u\ h\ s_t\ c_u\ s_r\ e^a\ c_a$	1	..	1	..	..	..	..	..	..	..	..	..
	t_h	..	..		..	..		..	..		..	..	
2,4	$r_u\ h\ c_u\ \ s_r\ e^a\ c_a$	1	1	3	3	..	8	2	2	9	2	2	7
	$t_h\ s_t$	1	..		5	..		4	1		1	2	
2,5	$r_u\ h\ s_r\ e^a\ c_a$	..	..	4	..	..	..	10	11	44	13	9	33
	$t_h\ s_t\ c_u$	4	..		..	..		12	11		7	4	

TABLE 5 (*continued*)

2,6	$r_u\ h\ e^a\ c_a$	1	..	3	..	..	..	5	9	29	6	9	25
	$l_h\ s_t\ c_u\ s_r$	2	..		..	..		9	6		4	6	
2,7	$r_u\ h\ c_a$	5	..	11	1	..	5	34	37	140	34	27	113
	$l_h\ s_t\ c_u\ s_r\ e^a$	4	2		3	1		39	30		21	21	
3,4	$r_u\ h\ l_h\ c_u\ s_r\ e^a\ c_a$	..	..	2	..	..	3	..	..	..	..	..	..
	s_t	..	2		..	3		..	..		..	..	
3,5	$r_u\ h\ l_h\ s_r\ e^a\ c_a$	..	..	..	..	..	..	..	..	..	..	..	..
	$s_t\ c_u$	..	..		..	..		..	..		..	..	
3,6	$r_u\ h\ l_h\ e^a\ c_a$	..	..	..	..	..	..	..	..	..	..	..	..
	$s_t\ c_u\ s_r$	..	..		..	..		..	..		..	..	
3,7	$r_u\ h\ l_h\ c_a$	..	..	..	..	..	..	..	..	1	..	..	2
	$s_t\ c_u\ s_r\ e^a$	..	..		..	..		..	1		..	2	
4,5	$r_u\ h\ l_h\ s_t\ s_r\ e^a\ c_a$	1	..	6	2	..	4	1	..	5	..	1	1
	c_u	5	..		2	..		2	2		..	..	
4,6	$r_u\ h\ l_h\ s_t\ e^a\ c_a$	1	..	4	..	..	..	2	4	11	1	..	1
	$c_u\ s_r$	2	1		..	..		4	1		..	..	

TABLE 5 (*continued*)

Diploids		I; 29 CULTURES			II; 25 CULTURES			I; 10 CULTURES			II; 10 CULTURES		
"Type"	Class	♀	♂	Total	♀	♂	Total	♀	♂	Total	♀	♂	Total
4,7	$r_u\ h\ l_h\ s_t\ c_a$	4	1	14	3	..	6	9	5	29	4	7	27
	$c_u\ s_r\ e^s$	8	1		2	1		6	9		9	7	
5,6	$r_u\ h\ l_h\ s_t\ c_u\ e^s\ c_a$	..	..	..	..	..	..	..	..	2	..	..	2
	s_r	..	..		..	..		2	..		1	1	
5,7	$r_u\ h\ l_h\ s_t\ c_u\ c_a$	1	..	6	..	..	4	8	4	37	10	5	37
	$s_r\ e^s$	5	..		4	..		12	13		11	11	
6,7	$r_u\ h\ l_h\ s_t\ c_u\ s_r\ c_a$	2	..	4	..	..	3	..	..	18	..	..	10
	e^s	2	..		3	..		11	7		3	7	
1,2,4	$r_u\ l_h\ s_t$	2	..	2	..	..	..	..	1	1	..	1	2
	$h\ c_u\ s_r\ e^s\ c_a$	..	..		..	..		..	..		1	..	

TABLE 5 (*continued*)

1,2,5	$r_u\ l_h\ s_t\ c_u$	1	1	2	..	..	..	1	..	3	..	..	1
	$h\ s_r\ e^s\ c_a$	..	..		..	..		1	1		..	1	
1,2,6	$r_u\ l_h\ s_t\ c_u\ s_r$	..	..	1	..	..	..	..	..	2	..	..	3
	$h\ e^s\ c_a$	1	..		..	..		2	..		2	1	
1,2,7	$r_u\ l_h\ s_t\ c_u\ s_r\ e^s$	2	..	2	..	..	..	..	2	9	2	..	8
	$h\ c_a$	..	..		..	..		3	4		..	6	
1,4,5	$r_u\ c_u$	..	..	..	..	..	..	..	1	1	1	..	1
	$h\ l_h\ s_t\ s_r\ e^s\ c_a$	..	..		..	..		..	..		..	..	
1,4,6	$r_u\ c_u\ s_r$	..	..	..	1	..	1	..	1	1	..	1	1
	$h\ l_h\ s_t\ e^s\ c_a$	..	..		..	..		..	..		..	..	
1,4,7	$r_u\ c_u\ s_r\ e^s$	..	..	..	..	..	1	1	1	8	1	1	5
	$h\ l_h\ s_t\ c_a$	..	..		1	..		4	2		2	1	
1,5,6	$r_u\ s_r$	..	..	..	..	..	..	..	1	1	1	..	1
	$h\ l_h\ s_t\ c_u\ e^s\ c_a$	..	..		..	..		..	..		..	..	
1,5,7	$r_u\ s_r\ e^s$	2	..	3	..	..	..	7	7	23	3	4	11
	$h\ l_h\ s_t\ c_u\ c_a$	1	..		..	..		7	2		2	2	

TABLE 5 (*continued*)

Diploids		I; 29 CULTURES			II; 25 CULTURES			I; 10 CULTURES			II; 10 CULTURES		
"Type"	Class	♀	♂	Total	♀	♂	Total	♀	♂	Total	♀	♂	Total
1,6,7	$r_u\ e^e$	1	..	1	..	..	..	1	2	5	2	4	8
	$h\ l_h\ s_t\ c_u\ s_r\ c_a$	..	..		..	..		..	2		2	..	
2,4,5	$r_u\ h\ c_u$	..	..	..	..	..	..	..	..	..	..	1	1
	$l_h\ s_t\ s_r\ e^e\ c_a$	..	..		..	..		..	..		..	..	
2,4,6	$r_u\ h\ c_u\ s_r$	..	..	..	..	..	..	1	..	1	..	1	1
	$l_h\ s_t\ e^e\ c_a$	..	..		..	..		..	..		..	..	
2,4,7	$r_u\ h\ c_u\ s_r\ e^e$	..	..	..	..	..	..	3	1	7	1	2	5
	$l_h\ s_t\ c_a$	..	..		..	..		..	3		1	1	
2,5,7	$r_u\ h\ s_r\ e^e$	..	..	..	..	..	1	4	8	21	2	2	12
	$l_h\ s_t\ c_u\ c_a$	..	..		1	..		4	5		4	4	

TABLE 5 (*continued*)

2,6,7	$r_u\ h\ e^s$	..	..	..	..	..	1	2	3	6	2	1	3
	$l_h\ s_t\ c_u\ s_r\ c_a$	..	..		1	..		1	..		..	..	
3,4,5	$r_u\ h\ l_h\ c_u$	..	..	2	..	..	..	..	..	..	..	..	..
	$s_t\ s_r\ e^s\ c_a$	..	2		..	..		..	..		..	..	
3,4,7	$r_u\ h\ l_h\ c_u\ s_r\ e^s$	..	..	1	..	..	..	..	..	..	..	..	..
	$s_t\ c_a$	..	1		..	..		..	..		..	..	
4,5,7	$r_u\ h\ l_h\ s_t\ s_r\ e^s$	..	..	1	..	..	..	..	..	.. 3	..	..	..
	$c_u\ c_a$	1	..		..	..		2	1		..	..	
4,6,7	$r_u\ h\ l_h\ s_t\ e^s$	1	..	1	..	..	..	..	..	..	..	..	1
	$c_u\ s_r\ c_a$	..	..		..	..		..	..		..	1	
1,2,4,5	$r_u\ l_h\ s_t\ s_r\ e^s\ c_a$	..	..	1	..	..	..	..	..	..	..	..	..
	$h\ c_u$	1	..		..	..		..	..		..	..	
1,2,4,6	$r_u\ l_h\ s_t\ e^s\ c_a$	..	..	..	..	..	..	..	2	2	..	..	..
	$h\ c_u\ s_r$	..	..		..	..		..	..		..	..	
1,2,5,7	$r_u\ l_h\ s_t\ c_u\ c_a$	..	..	..	..	..	1	..	..	..	..	..	..
	$h\ s_r\ e^s$	..	..		1	..		..	..		..	,,	

TABLE 5 (*continued*)

Diploids		I; 20 cultures			II; 25 cultures			I; 10 cultures			II; 10 cultures		
"Type"	Class	♀	♂	Total	♀	♂	Total	♀	♂	Total	♀	♂	Total
1,2,6,7	$r_u\ l_h\ s_t\ c_u\ s_r\ c_a$	..	..	..	..	..	..	..	..	..	..	..	2
	$k\ c^a$	..	..		..	..		..	..		1	1	
1,3,6,7	$r_u\ s_t\ c_u\ s_r\ c_a$	..	..	..	..	..	..	..	..	1	..	..	..
	$k\ l_h\ c^a$	..	..		..	..		..	1		..	..	
1,4,5,7	$r_u\ c_u\ c_a$	..	..	..	..	..	...	..	..	1	..	..	..
	$k\ l_h\ s_t\ s_r\ c^a$	..	..		..	..		1	..		..	..	
1,4,6,7	$r_u\ c_u\ s_r\ c_a$	..	..	..	..	..	..	..	..	1	..	..	..
	$k\ l_h\ s_t\ c^a$	..	..		..	..		1	..		..	..	

TABLE 5 (*continued*)

1,5,6,7	$r_u\ s_T\ c_a$	..	..	..	..	..	..	..	1	1	..	..	..
	$h\ l_h\ s_l\ c_u\ e^s$	..	..		..	..		..	..		..	..	
2,3,4,5	$r_u\ h\ s_l\ s_T\ e^s\ c_a$	..	..	..	..	1	1	..	..	..	..	..	..
	$l_h\ c_u$	..	..		..	..		..	..		..	..	
4,5,6,7	$r_u\ h\ l_h\ s_l\ s_T\ c_a$	..	..	1	..	..	..	..	..	..	..	..	..
	$c_u\ e^s$	1	..		..	..		..	..		..	..	
	Total	764	120	884	495	73	568	1379	1317	2696	1143	1080	2223

These facts are shown graphically by figure 1, which is from the recombination values for the first set of cultures. Ordinates represent the quotients of the recombination values, abscissae, the arrangement of genes along the chromosome as shown by the standard diploid map. The dotted lines give the respective probable errors for each region. This type of graphic treatment of crossing over data was previously used by MULLER

TABLE 6

Recombination values calculated from the data of tables 1, 2, 3, and 4.

REGION	TRIPLOID T	DIPLOID D	DIFFERENCE T-D	QUOTIENT T/D
CULTURES I				
s_s, h–M_h, D	12.3±0.88	14.0±0.34	− 1.7±0.94	0.88±0.07
M_h, D–m_a, c_u	25.0±1.16	6.9±0.25	18.1±1.19	3.62±0.21
m_a, c_u–S_b, b_z	11.8±0.86	7.4±0.25	4.4±0.90	1.59±0.13
S_b, b_z–H, e^s	8.9±0.49	10.9±0.19	− 2.0±0.52	0.82±0.05
H, e^s–M, c_a	20.9±0.91	32.6±0.37	−11.7±1.12	0.64±0.03
Total	78.9±1.98	71.8±1.19		
CULTURES II				
s_s, h–M_h, D	13.7±1.17	12.0±0.31	1.7±1.12	1.14±0.10
M_h, D–m_a, c_u	19.2±1.34	4.7±0.20	14.5±1.36	4.09±0.29
m_a, c_u–S_b, b_z	10.4±1.04	5.8±0.22	4.6±1.06	1.79±0.19
S_b, b_z–H, e^s	8.2±0.54	9.7±0.20	− 1.5±0.58	0.85±0.06
H, e^s–M, c_a	20.7±0.97	30.0±0.45	−19.3±1.07	0.69±0.03
Total	72.2±2.34	62.2±0.65		

(1925) in a study of the effects of X-rays. Since the quotients for cultures II are practically identical with those for cultures I (as is demonstrated by the table) it is unnecessary to publish graphs for the former.

Table 7 and figure 2 give the corresponding values for the second experiment. It is obvious that the results of the two experiments are in complete agreement. The effects observed are not, then, to be attributed

to crossover modifiers. This is shown also by the fact that standard values are obtained from the diploid controls.

It should be noted in passing that the strict correspondence between the two experiments is of some theoretical importance. In the first experiment all three chromosomes were marked; in the second experiment only one chromosome was marked, the other two being wild type. The fact

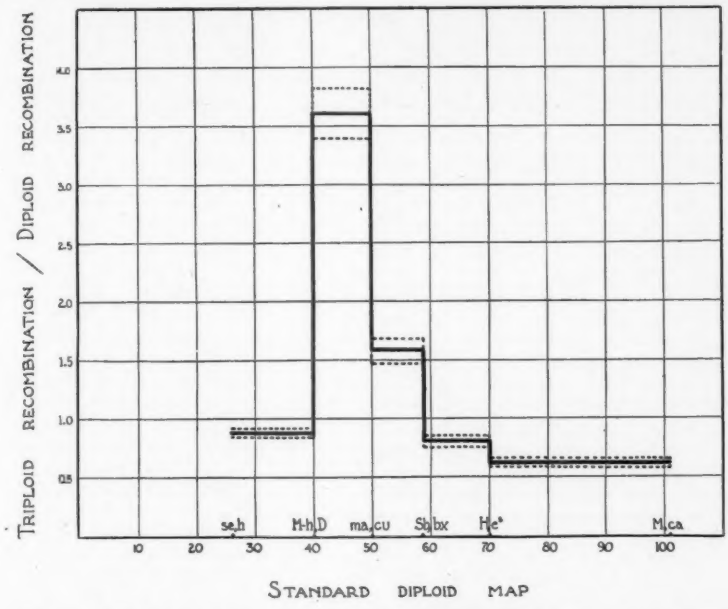

FIGURE 1.—The relation between triploid and diploid recombination along the chromosome. The data are from the first set of cultures of the first experiment. Ordinates represent the ratio of triploid recombination to diploid recombination; abscissae indicate the arrangement of the genes as shown by the standard diploid map. The dotted lines give the probable errors for the respective regions.

that the crossover behavior is the same in the two cases means, therefore, that the chromosomes cross over in the same manner whether they are marked or not. In other words, our assumption of page 220 is justified by the results, and we have definite evidence that crossing over is no more different between wild-type chromosomes than it is between two chromosomes one or both of which are marked. This has long been suspected, but has not previously been demonstrated.

If the assumption is made of free sister-strand interchange and the recombination values are corrected according to the discussion of page 206, we obtain the figures of table 8. A glance at this table will convince one that, although the recombination values are now somewhat different, their quotients still show the same regional differences and still have the same

TABLE 7

Recombination values calculated from the data of table 5.

CULTURES I

REGION	TRIPLOID T	DIPLOID D	DIFFERENCE T-D	QUOTIENT T/D
r_u–h	19.5±0.90	25.3±0.56	− 5.8±1.06	0.77±0.04
h–t_h	14.9±0.81	15.3±0.49	− 0.4±0.95	0.97±0.06
t_h–s_t	1.2±0.25	0.4±0.08	0.8±0.26	3.00±0.87
s_t–c_u	21.2±0.93	5.6±0.30	15.6±0.98	3.79±0.26
c_u–s_r	14.6±0.80	14.0±0.45	0.6±0.92	1.04±0.07
s_r–e^a	6.1±0.54	8.9±0.37	− 2.8±0.65	0.69±0.07
e^a–c_a	18.0±0.87	34.3±0.62	−16.3±1.07	0.52±0.03
Total	95.5±2.02	103.8±1.17		

CULTURES II

REGION	TRIPLOID T	DIPLOID D	DIFFERENCE T-D	QUOTIENT T/D
r_u–h	18.5±1.10	23.5±0.61	− 5.0±1.26	0.79±0.05
h–t_h	13.5±0.96	18.4±0.55	− 4.9±1.11	0.73±0.06
t_h–s_t	1.3±0.32	0.2±0.06	1.1±0.33	6.50±1.60
s_t–c_u	20.6±1.14	4.2±0.29	16.4±1.18	4.90±0.43
c_u–s_r	12.7±0.94	10.4±0.44	2.3±1.04	1.22±0.10
s_r–e^a	5.5±0.65	9.8±0.43	− 4.3±0.78	0.56±0.07
e^a–c_a	15.1±1.01	34.4±0.68	−19.3±1.22	0.44±0.03
Total	87.2±2.42	100.9±1.27		

general order of magnitude. The general conclusions remain unaltered, then, even if sister strands cross over as freely as non-sister strands.

An effect of age on crossing over might be expected to give significant differences between the recombination values for the first set of cultures and for the second set of cultures. Table 9 gives these differences listed separately for diploid and for triploid. The diploid mothers, especially of the first experiment, show a decrease of crossing over with age at central

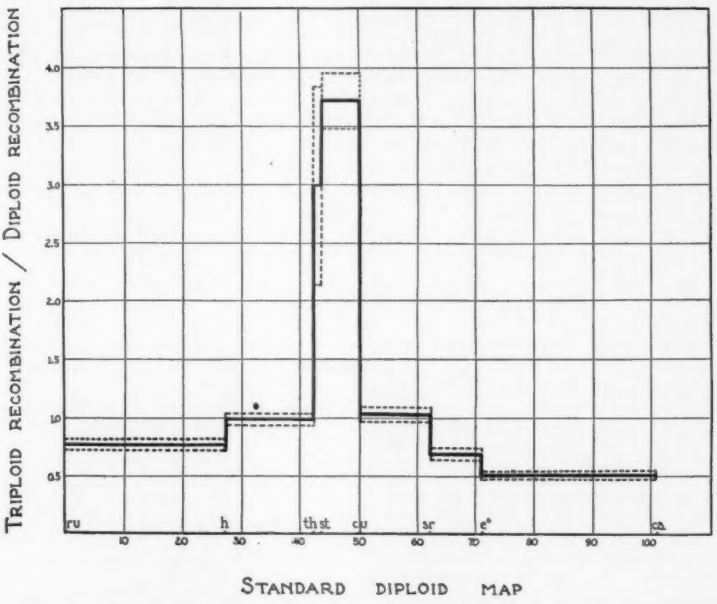

FIGURE 2.—The relations between triploid and diploid recombination along the chromosome. The data are from the first set of cultures of the second experiment. Ordinates and abscissae as in figure 1.

regions. These results are in agreement with those of PLOUGH (1921) and of BRIDGES (1927) for this chromosome. But there is no significant difference shown by the present data between the two sets of triploid cultures. This does not necessarily demonstrate the absence of an effect of age on crossing over in triploids. It is conceivable that such an effect exists, but that it is too delicate to be shown by the data, or that it does not coincide in its time relationships with that shown by diploids. At any rate it is obvious that there are differences between the age change in

diploids and in triploids. It is possible that this fact has some significant relation to the relative lengthening of the triploid chromosome in the center.

TABLE 8

Recombination values corrected on the basis of free sister-strand interchange.

CULTURES I

REGION	TRIPLOID	DIPLOID	REGION	TRIPLOID	DIPLOID
s_e,h–M_h,D	15.4±0.97	21.0±0.40	r_u–h	24.4±0.97	37.9±0.63
M_h,D–m_a,c_u	31.2±1.24	10.3±0.30	h–t_h	18.6±0.88	22.9±0.55
m_a,c_u–S_b,b_z	14.7±0.95	11.1±0.31	t_h–s_t	1.5±0.28	0.6±0.10
S_b,b_z–H,e^s	11.1±0.54	16.3±0.23	s_t–c_u	26.5±1.01	8.4±0.36
H,e^s–M,c_a	26.1±0.98	48.9±0.40	c_u–s_r	18.2±0.88	21.0±0.53
			s_r–e^s	7.6±0.60	13.3±0.44
			e^s–c_a	22.5±0.95	51.4±0.65
Total	98.5±2.15	107.6±0.75		119.3±2.20	155.5±1.32

CULTURES II

REGION	TRIPLOID	DIPLOID	REGION	TRIPLOID	DIPLOID
s_e,h–M_h,D	17.1±1.28	18.0±0.36	r_u–h	23.1±1.19	35.2±0.68
M_h,D–m_a,c_u	24.0±1.45	7.0±0.24	h–t_h	16.9±1.06	27.6±0.64
m_a,c_u–S_b,b_z	13.0±1.14	8.7±0.28	t_h–s_t	1.6±0.36	0.3±0.08
S_b,b_z–H,e^s	10.2±0.59	14.5±0.24	s_t–c_u	25.7±1.24	6.3±0.35
H,e^s–M,c_a	25.9±1.05	45.0±0.49	c_u–s_r	15.9±0.92	15.6±0.52
			s_r–e^s	6.9±0.72	14.7±0.51
			e^s–c_a	18.9±1.11	51.6±0.71
Total	90.2±2.55	93.2±0.75		109.0±2.61	151.3±1.43

It is the regional variation of crossing over along the chromosome with which we are primarily concerned. The regions of the third chromosome most affected by triploidy are the same regions most affected by temperature (PLOUGH 1921) and by X-rays (MULLER 1925). If the graphs of

figures 1 and 2 of the present paper are compared with the graphs for chromosome III of MULLER's paper, it will be seen that they are curiously alike. Indeed the only apparent difference is one of degree of effect; triploidy gives more extreme results than does the heavy X-ray dosage of MULLER. These similairites, as we shall see, have no direct significance. The results obtained by the subjection of diploids to temperature and to X-rays are correlated with the distance of the region involved from the spindle-fibre attachment. A comparison of recombination values for

TABLE 9

Age change in crossing over as shown by the recombination for cultures I minus the recombination for cultures II. From tables 6 and 7.

REGION	TRIPLOID	DIPLOID	REGION	TRIPLOID	DIPLOID
s_c,h–M_h,D	−1.4±1.46	2.0±0.46	r_u–h	1.0±1.42	1.8±0.83
M_h,D–m_a,c_u	5.8±1.77	2.2±0.32	h–t_h	1.4±1.26	−3.1±0.74
m_a,c_u–S_b,b_z	1.4±1.35	1.6±0.33	t_h–s_t	−0.1±0.41	0.2±0.10
S_b,b_z–H,e^a	0.7±0.73	1.2±0.28	s_t–c_u	0.6±1.47	1.4±0.42
H,e^a–M,c_a	0.2±1.33	2.6±0.58	c_u–s_r	1.9±1.23	3.6±0.63
			s_r–e^a	0.6±0.84	−0.9±0.57
			e^a–c_a	2.9±1.33	−0.1±0.92

chromosomes I, II, and III from such treated diploid females shows, so far as the data go, that the effect is greatest at the region of spindle-fibre attachment and decreases progressively from that region. This, as we have seen, is true also of chromosome III of triploids.

But when we consider chromosome I of triploids, and compare the data with those for chromosome III, we find that the results are exactly opposite in the two chromosomes with respect to distance from the spindle-fibre attachment. BRIDGES and ANDERSON (1925) found the following variation along chromosome I: crossing over in the extreme left end (from yellow, 0.0 to bifid, 6.9) was twice as high as in the diploid controls; but in regions to the right of bifid it was only about one-half as high. The right end of the chromosome is the region of spindle-fibre attachment. In the first chromosome, then, the increase is at the distal end of the chromosome with respect to the spindle-fibre attachment. If the data of BRIDGES and ANDERSON are plotted in the same manner as the data of the present paper

a curve is obtained similar in form to one-half the curve of figure 2. This would seem off-hand to correspond with the fact that the X-chromosome is rod shaped, whereas the third chromosome is V-shaped (that is, consists of two rods fastened together). But the maximum of the curve for chromosome III falls at the region of spindle-fibre attachment, and the maximum of the corresponding curve for chromosome I falls at the opposite end of the chromosome with respect to the attachment.

However, although the differences between triploid and diploid crossing over are not to be explained on the basis of a differential effect depending upon distance from the spindle-fibre attachment, it is a fact that a correlation exists between the lengthening or shortening of the triploid map and the spacing of the genes in the chromosomes as exhibited by the diploid map. Regions in which genes are clumped in the diploid, that is, in which there is relatively little crossing over between successive genes, are longer in the triploid; and, conversely, regions in which genes are far apart in the diploid, that is, in which there is relatively much crossing over between successive genes, are shorter in the triploid. This is true both for chromosome I and chromosome III. It seems reasonable to suppose, therefore, that triploid crossing over gives us a more dependable measure of the actual physical distance between genes than does diploid crossing over.

MULLER, ALTENBURG, and PAINTER (MULLER and ALTENBURG 1928, MULLER and PAINTER 1929) have recently reached conclusions of much interest in this connection from data of an entirely different nature. In a study of translocations produced in the diploid by exposure to X-rays these investigators find that regions in which genes are normally clumped are seen on translocation to be longer than the genetic results had previously indicated, whereas regions in which genes are normally sparse are seen to be shorter. That is, the scale of our diploid maps varies from region to region. If the contention of the present paper is justified, the scale of the triploid maps varies less than the scale of the diploid maps.

It may be stated incidentally that incomplete data upon crossing over in the second chromosome of triploids give results similar in direction but not so extreme in absolute value as those for the third chromosome of triploids.

The cytological studies of triploid Drosophilas by METZ (1925) led him to suggest that the peculiar genetic results obtained for the X-chromosome by BRIDGES and ANDERSON were a reflection of the observed anomalous behavior of the X-chromosomes. "The autosomes appear to associate uniformly throughout their length, but the X-chromosomes seem to behave differently. In most figures the latter are closely associated for about

half their length, and then diverge, finger-like as shown by the drawings. This may be due to a precocious separation of the homologues at one end, preceded by a state of close association throughout." The fact that chromosome III exhibits an even greater regional differentiation than the X would indicate that the correlation observed by METZ was of no direct causal significance.

CORRECTION CURVES AND MAPS

Correction curves may be constructed for the triploid data according to the method of BRIDGES (BRIDGES and MORGAN 1923) for the diploid, giving by a process of extrapolation the correction for double crossing over for the region between any two successive genes. The data of the first experiment are hardly amenable to such treatment since two crosses were necessary in covering the chromosome. Correction curves from the trip-

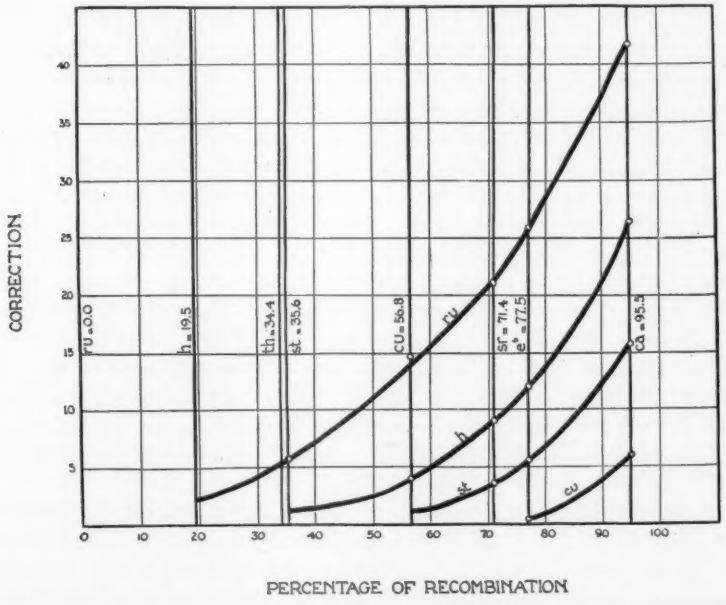

FIGURE 3.—Correction curves to give map distances for the third chromosome of triploids. The data used are from the first cultures of the second experiment. Ordinates represent the correction to be added to the respective recombination percentage to give the crossover value. The difference between the abscissae of two *succeeding* vertical lines represents the recombination value for the corresponding genes.

loid data of the second experiment are given in figure 3. Corresponding correction curves from the diploid data were practically identical with those published by BRIDGES and MORGAN (1923) and are therefore not given here. The ordinates of figure 3 represent the corrections to be added to the respective recombination percentages in order to give the correct crossover values, that is, the map distances. The difference between the abscissae of two succeeding vertical lines gives the recombination between the corresponding genes; this does not apply to genes separated by one or more mutant genes of the cross. The open circles of each curve show for the indicated gene the corrections calculated from the experimental data. These curves may be extended smoothly from the last circle toward the base line giving the approximate correction between successive loci. These corrections make no pretense to a high degree of accuracy. They will, however, facilitate somewhat the prediction of crossover values for chromosome III in triploid Drosophilas.

The one suggestive difference between these curves and those for the diploid depends upon the fact that the triploid curves begin with the first open circles at higher levels, at least for the ends of the chromosome. If this difference is significant it would indicate that double crossing over is higher in the triploid than in the diploid at the ends of the chromosome, in spite of the fact that the triploid recombination values are about half the diploid values. In other words, the reduction in crossing over at the ends of the triploid chromosome is dependent upon a considerable reduction in single crossing over, and exists in spite of a possible increase in double crossing over.

For the center of the chromosome there is no detectable difference between the corrections for triploid and for diploid. So far as the data go, therefore, they would indicate that there is a very marked increase in single crossing over in central regions of the triploid chromosome which entirely overshadows an apparent equality in double crossing over. These differences, however, should not be emphasized for it is not certain that they are significant.

Corrected maps for the triploid and diploid may be compared in figure 4. These maps are constructed similarly from the data of the first cultures of the second experiment. The values for the diploid are slightly higher than those of the standard diploid map, but this is to be expected since in the present experiments data from young and old females were segregated, which is not the case in ordinary crossover work. The genes used in the first experiment were omitted since the correction curves for these crosses are less accurate than are those of figure 3.

RECURRENT AND PROGRESSIVE DOUBLE CROSSOVERS

It is of interest to consider the relative occurrence of recurrent and progressive double crossovers. Obviously only the results of the first experiment are of value in this connection. When the proper data are selected

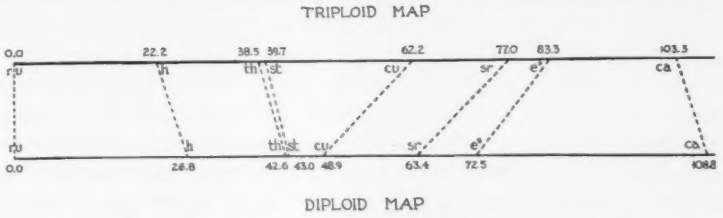

FIGURE 4.—A comparison of triploid and diploid maps for the third chromosome. The data are from the first cultures of the second experiment. Dotted lines connect the same genes.

from tables 1 and 3, table 10 is obtained. This table includes not only the simple double crossovers, but also the four triples of table 1, for a triple may be regarded as a succession of two double crossovers.

TABLE 10

A comparison of recurrent and progressive double crossovers. The data are taken from tables 1 and 3.

	RECURRENT	PROGRESSIVE	DIFFERENCE
Cultures I	43	43	0
Cultures II	20	26	6
Total	63=47.7%±2.9%	69=52.3%±2.9%	6=4.5%±4.1%

It is clear that the recurrent doubles and the progressive doubles are equal in number. It follows therefore that the chromosomes taking part in the first of two crossovers have no influence on the chromosomes taking part in the second. Or more accurately, when we recall that six crossover strands are present instead of three, one of the two strands which have crossed over at a first level crosses over at a second level as freely with a strand from the third chromosome as with a strand from the original two chromosomes. This would indicate that synapsis involves all three chromosomes equally. It would indicate further that the interference effects in triploids are not appreciably different for two successive crossovers in the same two strands than they are for two successive crossovers involving three strands. For if interference in the first case were greater, as one might

possibly be led to expect on *a priori* grounds, it would follow that progressive doubles would be more frequent than recurrent doubles. The equality of the two types of double crossovers observed here is quite in accord with the similar results of BRIDGES and ANDERSON (1925) for the X chromosome. These investigators record 15 recurrent and 10 progressive doubles. This would make a sum total of 78 recurrents and 79 progressives observed to date.

Coincidence values are not, strictly speaking, comparable in diploid and triploid. For coincidence is defined as the ratio of the actual percentage of doubles in two given regions to the expected percentage of doubles in these regions. Now the percentage of doubles expected as a matter of chance is obtained by multiplying together the crossover percentages of the two regions involved. It is obvious that this product represents the expectation of actual doubles (as exhibited by the emerging chromosomes) only in case two strands are in synapsis. It cannot be the expectation in four-strand crossing over, for the two separate crossings over do not necessarily involve the same single strand as central section. Still less is it the expectation in six-strand crossing over. Of course one may form the product irrespective of its significance and divide the observed percentage of doubles by it, thus obtaining an expression for "coincidence." This expression has no direct meaning in terms of the mechanism involved, but it is of some value in prediction and in comparison. "Coincidence" values are therefore given in table 11. Only data from the first experiment are

TABLE 11

"Coincidence" values for triploid and diploid derived from the data of the first experiment.

	CULTURES I		CULTURES II	
REGIONS	TRIPLOID	DIPLOID	TRIPLOID	DIPLOID
1,2	0.87	0.39	0.77	0.35
1,3	0.87	0.94	0.89	0.93
1,4	0.81	0.99	1.69	1.08
2,3	1.17	1.00	0.76	1.54
2,4	0.80	1.01	0.80	1.36
3,4	1.02	0.77	0.37	0.72
4,5	0.86	0.45	0.94	0.34

included here because the actual number of doubles cannot be directly observed in the second experiment.

It is immediately apparent that the values for the triploid are not uniformly higher than are those for the diploid. In this respect the results for the third chromosome are not in agreement with those of BRIDGES and ANDERSON (1925) for the X chromosome.

THE SPINDLE-FIBRE ATTACHMENT

The attachment of the spindle fibre to the third chromosome is seen in cytological preparations to be near the center of the chromosome at the apex of the V. Moreover the genetic evidence has long indicated that this point is somewhere between scarlet and curled near peach (48.0, standard diploid map). Evidence is now at hand from the recent paper of MULLER and PAINTER (1929), from the unpublished work of DOBZHANSKY, and from the data of the present paper to locate this attachment more accurately.

Certain individuals of the second experiment which were not classified in table 5 according to the characters which they displayed may now be considered. They include the so-called equational exceptions, that is, individuals which have received two third chromosomes from their mothers, these two chromosomes being derived wholly or in part from sister strands. Such exceptions will, of course, be detected only in case they are equational for one of the recessive mutant genes present in the mother in single dose. They will necessarily occur only among the triploid, intersex, and supermale offspring, since these alone have the requisite number of third chromosomes. Unfortunately the characters roughoid, hairy, and thread could not be classified with certainty in these experiments in intersexes; hence they were disregarded in formulating table 12.

If we count the number of times each gene is represented in this table we have the following graded series: s_t, 6; c_u, 6; s_r, 13; e^s, 16; c_a, 26. Now the number of representations of each gene expected as a matter of chance is 73.7. This figure is obtained as follows. If we consider any given locus, for example the scarlet locus, it is obvious that there are present in the six strand stage two scarlet sister genes and four normal allelomorphs. If in the formation of the diploid egg chance alone determines which two of these six allelomorphs pass to the given pole, 2/30, that is, 1/15, of the diploid eggs will contain two scarlet genes. Now the total number of eggs diploid for the third chromosome which are to be considered is represented by the sum of the total numbers of triploids, female intersexes, male intersexes, and super males; this sum is 1106. One fifteenth of this sum is, then, 73.7. The fact

that the representations fall much below expectation would indicate that the distribution is not a chance distribution. Another obvious relationship is that the genes form a graded series according to their distance from the center of the chromosome.

TABLE 12

Equational exceptions for the second experiment.

CLASS	TRIPLOIDS	♀ INTERSEXES	♂ INTERSEXES	SUPERMALES
S_l	1	2	1	1
$S_l\ e^s$	..	..	..	1
$C_u\ S_r\ e^s$	..	..	1	..
$C_u\ S_r\ e^s\ C_a$	..	2	3	..
S_r	..	..	1	..
$S_r\ e^s$	..	1	..	..
$S_r\ e^s\ C_a$	1	1	3	..
e^s	1	1	..	..
$e^s\ C_a$	..	1	..	..
C_a	1	10	5	..

A similar graded series of representations of genes in equational exceptions from triploids was found by BRIDGES and ANDERSON (1925) for the X chromosome. For this chromosome the number of equationals is lowest for the right end and increases progressively toward the left end. It is assumed by these investigators that separation of sister genes always occurs at the spindle fibre. Equational exceptions at loci not at the region of spindle-fibre attachment depend, then, upon crossing over and a resulting association of sister genes which would otherwise have been separated. The amount of crossing over would increase, obviously, with the distance of the locus from the spindle fibre. The attachment of the fibre to the X chromosome is therefore supposedly at the right end. The same conclusion had previously been reached by a similar consideration of the equational exceptions occurring in experiments with attached X chromosomes (ANDERSON 1925, L. V. MORGAN 1925 and unpublished work of STURTEVANT).

According to the same argument the spindle-fibre attachment to the third chromosome could not be to the right of curled. The fact that in no case do scarlet and curled appear in the same equational exception of table 12 would seem to demonstrate that the attachment is between these two genes. It may be stated that for those equational exceptions in which roughoid, hairy, and thread were readily classified, that is, in the triploids and supermales, the number of representations is as follows: r_u, 6; h, 4; l_h, 3; s_l, 3; Although these numbers are low they indicate, as expected, that the spindle fibre is not to the left of scarlet. The present data, therefore, place the attachment at the center of chromosome III between scarlet and curled. This location was previously suggested by the data of BRIDGES from preliminary crosses with triploid females designed to determine the attachment (MORGAN, STURTEVANT, and BRIDGES 1925).

These conclusions regarding spindle-fibre attachment are in complete agreement with those recently reached by MULLER and PAINTER (1929) and by DOBZHANSKY (unpublished) from a study of translocations. MULLER and PAINTER report a translocation in which slightly less than half of the third chromosome has broken away and has become attached to the end of the second chromosome. The cytological preparations demonstrate clearly that this break is just to the left of the spindle-fibre attachment. The genetic position of the break is, moreover, slightly to the left of the center of the linkage map half way between scarlet and pink—that is, at about 46 on the diploid map. This evidence, in conjunction with the evidence from triploids discussed above, would then place the attachment in a region limited by loci 46 and 50 of the standard diploid map.

The work of DOBZHANSKY offers further confirmation. In a study of translocations of pieces of the third chromosome to the fourth chromosome two cases were found in which the break occurred very near the spindle fibre, one just to the left of the fibre, and one to the right. These cases make possible the location of the spindle fibre with respect to the mutant genes marked. For from a study of the crossing over relationships it is clear that scarlet is to the right of the first break, and curled to the left of the second. The appearance of the chromosomes, as well as the crossover relations, gives DOBZHANSKY an opportunity to estimate the distances from these genes to the spindle fibre the attachment of which he accordingly places between loci 46 and 48.

DOBZHANSKY'S results are of further interest in connection with crossing over in triploids, for they show that, for central regions of the third chromosome, distances between genes are much longer than our standard diploid maps would indicate. This is obviously in agreement with the similar

results of MULLER and PAINTER (1929) and with the conclusions of the present paper (see page 240).

CELL SIZE AND CROSSING OVER

That there are marked differences between triploid and diploid crossing over cannot be questioned, and it would seem clear that triploid crossing over more nearly represents the actual distance between genes than does diploid crossing over, but the cause of the differences remains quite obscure. So far as we know, the crossing over system in triploid Drosophilas differs from that in the diploids in two respects. In the first place there are present six crossing over strands instead of four, and secondly the triploid cell is much larger than the diploid cell. The former follows from the data of BRIDGES and ANDERSON (1925), and the latter has been demonstrated by DOBZHANSKY (1929).

It is conceivable that the presence of six crossing over strands gives rise *per se* in some manner to the differences; they are not, however, as has been shown, the mere direct concomitant of sister-strand crossing over. On the other hand it seems plausible that marked alterations in the size of the cell-components might involve alterations in the relations of chromosomes to cytoplasm, and thus result in crossover variations.

There seem to be two immediate methods of attacking these problems. We may reduce the number of crossover strands in the triploid cell, and we may (possibly?) alter the size of the diploid cell. A reduction of the number of crossover strands is brought about readily by the proper use of crossover reducers. Crosses of this type are now well under way, but are not ready to report.

It was hoped, in the second place, that the diploid cell size of the mutant giant might be comparable to that of the normal triploid cell. The work of GABRITSCHEVSKY and BRIDGES (1928) had shown that giant and non-giant females from giant stock do not differ genetically and that the crossover values from these two types are normal and equal. It was known further from measurements of the chromosome plates published by these investigators that the chromosomes of giant females do not differ in length, and presumably also not in thickness, from ordinary diploid chromosomes. If giant cells proved to be larger than non-giant cells we should, therefore, have the altered diploid system desired.

Accordingly enough measurements were made of the cell size of giant and of non-giant females from giant-bobbed[11]-attached-X stock to detect any differences. These measurements were made with the assistance of Mr. IRWIN SPRITZER using the technique of DOBZHANSKY. Bristle counts

were made in three specific different regions of the wing. Since it is known that each cell of the wing is provided with a single bristle, it follows that the cell size is inversely proportional to the number of bristles per area. Table 13 gives these results as well as the results for normal female, normal male, and normal triploid taken from the paper of DOBZHANSKY.

TABLE 13

Number of bristles per 0.01 mm² in three given regions of the wing. The figures for 2N ♀, ♂, and 3N ♀ are from DOBZHANSKY. The giant females and non-giant females were from giant bobbed[11]-attached-X stock.

	REGION I	REGION II	REGION III
giant ♀	56.02±0.58	63.12±0.61	57.37±0.50
non-giant ♀	59.82±0.66	67.04±0.56	61.38±0.52
difference	3.80±0.88	3.92±0.83	4.01±0.72
2N ♀	50.76±0.22	61.01±0.31	55.67±0.29
♂	57.42±0.35	70.46±0.36	63.02±0.33
3N ♀	36.70±0.22	44.47±0.27	41.35±0.25

These figures demonstrate a difference which is just significant between the cell size of the giant female and that of the non-giant female; the two types are from the same culture and are presumably of identical composition. DR. BRIDGES informs me that preparations of the ovaries of giant females show rare cells diploid in composition and distinctly larger in size than the surrounding cells. It is possible that similar occasional large cells are present in the wing and account for the slightly larger average size of cells of the giant as compared with the non-giant, as shown in the table. The cells of the latter are somewhat different in size from those of the normal diploid female, but this is no doubt to be explained on the basis of the presence of different modifiers in the two stocks.

At any rate it is perfectly clear that the size of the triploid cell is of an entirely different order of magnitude from the size of cells of giant diploid females. It follows, then, that the counts from giant stock have no relation to the work on crossing over in triploids. They have been mentioned here because they have some incidental interest of their own. It would seem that, since the size of giant and of non-giant cells is not very different, the

distinctly greater size of the giant fly is dependent (at least partially) upon a greater number of cells. The rare presence of large cells in the ovaries, however, renders such a conclusion tentative until the matter is investigated further.

CONCLUSIONS

(1) In *Drosophila melanogaster* crossing over in the triploid shows marked regional differences along the third chromosome from crossing over in the diploid. Crossing over at the ends of the chromosome is slightly more than one-half as great in the triploid as in the diploid; and as we pass from either end of the chromosome to the center the relative amount of crossing over in the triploid increases continuously until we reach a maximum at the center, for which region triploid crossing over is more than three times as great as diploid crossing over.

(2) A comparison of these results with those of BRIDGES and ANDERSON for the X chromosome of triploids results in the conclusion that the variations are not correlated with the distance of the region from the spindle-fibre attachment.

(3) They are correlated, however, with the regional spacing of the genes as represented by the diploid maps. Regions in which genes are closely spaced on the diploid maps are lengthened in the triploid; and regions in which genes are far apart on the diploid maps are shortened in the triploid.

(4) It is probable, therefore, that the triploid maps represent more accurately than the diploid maps the actual spacing of the genes along the chromosomes.

(5) The data do not show any effect of age upon crossing over in the third chromosomes of triploids, although the usual effect is shown by the diploid controls.

(6) Crossing over takes place between two wild-type chromosomes to the same extent that it does between two chromosomes one or both of which are marked by mutants.

(7) For chromosome III the number of recurrent double crossovers is equal to the number of progressive double crossovers. It follows that the strands taking part in the first crossing over do not in any way determine which strands take part in the second.

(8) The data of the present paper, in conjunction with those of MULLER and PAINTER and of DOBZHANSKY, place the spindle-fibre attachment of chromosome III in the center of the chromosome at some point between loci 46 and about 48 of the standard diploid map.

(9) In attempts to determine the cause of the differences between diploid and triploid crossing over, measurements were made of the cells of females from giant stock. The average cell size of giant females is slightly greater than that of non-giant females from the same stock; it does not approach, however, the size of cells of triploid females.

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THE INHERITANCE, INTERACTIONS AND LINKAGE RELATIONS OF GENES CAUSING YELLOW SEEDLINGS IN MAIZE*[1]

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[1] The data on which this paper is based were obtained in connection with the corn breeding program conducted by the Office of Cereal Crops and Diseases, UNITED STATES DEPARTMENT OF AGRICULTURE and the Farm Crops Section, IOWA AGRICULTURAL EXPERIMENT STATION, cooperating.

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INTRODUCTION

Two genes affecting the production of yellow pigment in maize already have been reported by LINDSTROM (1925). He designated them as l_1 (luteus) and l_2. The present paper reports two additional genes which have been called l_3 and l_4. The inheritance of both of these genes, their interactions with a number of other chlorophyll factors, and the linkage relations of one of them are reasonably well established.

GENES l_1 AND l_2

Gene l_1 was reported first by LINDSTROM in 1917. This gene governs the formation of a distinct yellow pigment but has no effect upon the green pigments. The action of l_1, therefore, may be observed only in such genotypes as suppress the development of the green pigments either partially or entirely. With the proper genotype the action of l_1 may be observed both in seedlings and mature plants. In the latter, l_1 is best known in the japonica type of striping. With l_1 such japonica plants have alternate stripes of green and yellow, whereas if L_1 is present the stripes are green and white. With the proper genotype, l_1 was found by LINDSTROM to occur as a pure-yellow or a virescent-yellow seedling. He has summarized the interactions of l_1 (LINDSTROM 1925) with the typical seedling genes as follows:

L_1W_1V,	green
l_1W_1V,	green
L_1W_1v,	virescent-white
L_1w_1V,	white or albino
L_1w_1v,	white or albino
l_1W_1v,	virescent-yellow
l_1w_1V,	pure yellow
l_1w_1v,	pure yellow

The interaction between l_1 and the albino genes w_2, and w_3 is similar to the interaction of this gene with w_1.

Gene l_2 also was reported by LINDSTROM (1925). It differs markedly from l_1 both in its appearance and in its interaction with other known chlorophyll genes. The yellow seedlings are deeper in color than those produced by l_1. Gene l_2, unlike l_1, produces yellow seedlings by itself and is lethal in the homozygous recessive condition. In this respect its action is similar to the various albino genes. LINDSTROM found that whereas l_1 gave a 12:3:1 interaction with W_1w_1, l_2 exhibited a 9:3:4 relation as follows:

9	L_2W_1	green
3	l_2W_1	yellow
3	L_2w_1	white
1	l_2w_1	white

This 9:3:4 relationship also held for the albino genes W_2 and W_3.

Linkage studies (LINDSTROM 1925) with l_1 and l_2 have placed them about 35 units apart in the *R-G* linkage group. The order of the genes on this chromosome as determined by LINDSTROM (1925) is L_1-*R*-W_2L_2. L_1 and *R* are linked very closely if not completely.

GENES l_3 AND l_4

The l_3 and l_4 genes were first observed in 1923 in the seedling progenies from plants self-pollinated for the first time during the previous summer. The yellow seedlings produced by these two genes are indistinguishable from those produced by l_2. The parent plant from which l_3 was obtained came from the commercial variety Iodent, and l_4 came from Clark Yellow Dent. When first isolated l_3 and l_4 were associated with the chlorophyll defect, iojap (i_j), the inheritance of which has been reported (JENKINS 1924). Both of these yellow factors, in their interaction with i_j, produced some white-striped-yellow seedlings. When i_j was reported it was mentioned that in some pedigrees segregations of green, white-striped-green, and yellow-striped-green seedlings were obtained, while in other pedigrees the classes were green, white-striped-green, yellow, and white-striped-yellow seedlings. At that time it was not known whether this was due to two different factors for striping or two different factors for yellow. It is now evident that it was due to different factors for yellow seedlings.

INHERITANCE OF l_3 AND OF l_4

The yellow seedlings produced by both l_3 and l_4 are simple Mendelian recessives to the normal green. Both genes when first isolated appeared to be linked with lethal or semi-lethal factors which caused a deficiency in the yellow seedling class and gave distorted 3:1 ratios. Satisfactory 3:1 ratios were not obtained until these cultures had been outcrossed and the disturbing factors eliminated. Critical evidence that the yellow seedlings produced by both l_3 and l_4 were simple recessives was obtained, however, from the proportion of homozygous (*LL*) and heterozygous (*Ll*) green plants in progenies from self-fertilized plants which were heterozygous for yellow seedlings, and from the proportion of homozygous and heterozygous plants in progenies resulting from crosses of plants heterozygous for either

$L_3 l_3$ or $L_4 l_4$ with plants of unrelated stocks. In the first case the proportion of homozygous to heterozygous plants should be 1:2 and in the second case 1:1, if a single Mendelian factor is concerned.

Seedling progenies of the original plant and of 150 other plants heterozygous for $L_3 l_3$ have been grown. The progenies contained 12,415 non-yellow and 3,377 yellow seedlings. This is far removed from a 3:1 ratio, Dev.÷P. E. being 15.6. A closer approximation to a 3:1 ratio was obtained from 71 F_2 progenies of two crosses between the stock heterozygous for $L_3 l_3$ and unrelated stocks. In this case totals of 5,056 non-yellow and 1,576 yellow seedlings were obtained. Here the Dev.÷P. E. was 3.4, still not a particularly good fit.

More critical evidence that only one factor was concerned in the production of l_3 yellow seedlings was obtained from the proportion of homozygous $L_3 L_3$ and heterozygous $L_3 l_3$ normal green plants in progenies segregating for these yellow seedlings and in the F_1 progenies of the cross $L_3 l_3 \times L_3 L_3$. Among 129 self-pollinated green plants from segregating progenies 48 were homozygous and 81 were heterozygous. In this case Dev.÷P. E. was 1.4, indicating a good fit. Among 151 self-pollinated plants from F_1 progenies of the cross $L_3 l_3 \times L_3 L_3$, 80 proved to be homozygous and 71 heterozygous. The Dev.÷P. E. was 1.1 in this case. From these data it seems safe to conclude that the l_3 yellow seedlings are the result of the action of a single factor.

Progenies have been grown from the original plant and from 75 additional plants heterozygous for $L_4 l_4$. These progenies contained a total of 7,672 non-yellow and 2,045 yellow seedlings. Here, again, there is a large deficiency in the yellow seedling class and very poor agreement with the expected 3:1 ratio. The Dev.÷P. E. in this case was 13.3. Much better ratios were obtained among the F_2 and F_3 progenies of a cross between the l_4 stock and an unrelated stock. Progenies from 35 heterozygous F_2 and F_3 plants produced 3,925 non-yellow and 1,231 yellow seedlings. In this case Dev.÷P. E was 2.8, indicating fair agreement between the observed and expected numbers.

Fifty-eight normal green plants in progenies segregating for l_4 yellow seedlings were self-pollinated. The progenies from these plants indicated that 25 of them were homozygous $L_4 L_4$ and 33 were heterozygous $L_4 l_4$. The Dev.÷P. E. for the expected 1:2 ratio was 2.4. Data on the progenies from 36 self-pollinated F_1 plants of the cross $L_4 l_4 \times L_4 L_4$ indicated that 18 were homozygous and 18 were heterozygous. These are exactly the expected numbers on the basis of a 1:1 ratio. It may be concluded, therefore,

that the l_4 yellow seedlings are the result of the action of a single genetic factor.

PROOF THAT l_1, l_2 l_3 AND l_4 ARE DIFFERENT GENES

LINDSTROM (1925) has shown that l_1 and l_2 are different. Crosses between l_1 and l_3 has demonstrated that these genes are different. The F_1 plants of such a cross are normal green, and under the proper conditions dihybrid ratios of green to yellow seedlings are obtained in F_2. No crosses have been obtained between l_1 and l_4. However, the phenotypic differences between the yellow seedlings produced by these two genes, the different interactions of the respondible genes with other chlorophyll genes, and the fact that they have different linkage relations leaves little doubt but that they differ genetically.

Genes l_2, l_3 and l_4 are phenotypically alike, and similar in their interactions with other chlorophyll genes with which they have been tested. All possible combinations have been made between plants heterozygous for these genes. The F_1 plants in each case have been normal green and 9:7 ratios of green to yellow seedlings have been obtained in the F_2 generation. In the case of the cross $L_2l_2 \times L_4l_4$ the 9:7 ratios were modified by linkage as these genes are located on the same chromosome.

INTERACTION OF THE FOUR GENES PRODUCING YELLOW SEEDLINGS WITH CERTAIN OTHER GENES FOR CHLOROPHYLL DEFICIENCY

Dihybrid ratios involving l_1 and i_j

Gene l_1 interacts with i_j in a manner similar to its interaction with j as described by LINDSTROM (1918). The iojap character may be observed in the seedlings and for this reason is a better character to work with than japonica. Self-pollinated plants heterozygous for $L_1l_1\,I_jI_j$ give the following dihybrid ratio in F_2:

9 $L_1\,I_j$ 3 $l_1\,I_j$	green plants
3 $L_1\,i_j$	white-iojap plants
1 $l_1\,i_j$	yellow-iojap plants

The white-iojap plants are so named because they have alternate stripes of green and of white tissue and the yellow-iojap plants because they have alternate stripes of green and of yellow tissue.

A summary for these two factors is given below:

	L_1I_j and l_1I_j	L_1i_j	l_1i_j
Observed	5969	1420	503
Expected	*5919*	*1480*	*493*
Deviations	+50	−60	+10
$\chi^2=3.06$		P=0.22	

Progenies have been grown from 33 plants heterozygous for both of these factors which were backcrossed to the double recessive (l_1i_j). Ratios of 2 green:1 white-iojap:1 yellow-iojap seedlings were expected. The data from these progenies are summarized below:

	L_1I_j and l_1I_j	L_1i_j	l_1i_j
Observed	3371	1545	1528
Expected	*3222*	*1611*	*1611*
Deviations	+149	−66	−83
$\chi^2=13.87$		P=0.001	

The poor fit in this case is due to a shortage of iojap plants, and in no way indicates linkage as there is very close to a 1:1 ratio of L_1 and l_1 plants among those recessive for i_j.

Dihybrid ratios involving l_2, l_3 or l_4 and i_j

When plants of the genetic composition $L_3l_3\,I_ji_j$ were self-pollinated and their progenies grown, they contained 4 classes of seedlings in the proportions expected on the basis of independent inheritance. The phenotypic and genotypic description of the seedling classes obtained is given below.

9 $L_3\,I_j$ green
3 $L_3\,i_j$ white-iojap
3 $l_3\,I_j$ yellow
1 $l_3\,i_j$ white-striped-yellow

The double recessive class is unusual and is very distinct in progenies giving a good clear-cut segregation for iojap.

A summary of the data on 31 progenies from plants heterozygous for these two factors is given below.

	L_3I_j	L_3i_j	l_3I_j	l_3i_j
Observed	2309	725	516	136
Expected 9:3:3:1	*2073*	*691*	*691*	*230*
Deviations	+236	+34	−175	−94
$\chi^2=116.4$			P=very small	
Expected (3:1 *segregation for i_j in the L_3 and l_3 classes*)	*2276*	*758*	*489*	*163*
Deviations	+33	−33	+27	−27
$\chi^2=7.88$			P=0.02	

The deviations from the numbers expected on the basis of a 9:3:3:1 ratio are large. They are due chiefly to the deficiencies in both the l_3 classes however. On the basis of the independent inheritance of these two factors there should be a 3:1 segregation for non-iojap and iojap plants in the L_3 and in the l_3 classes. The deviations computed on this basis are much smaller, P having a value of 0.02. The poor fit is due, however, to deficiencies in the recessive classes and not to linkage.

Data not included in this report show that l_2 and l_4 give interactions with i_j similar to those of l_3.

Dihybrid ratios involving l_2, l_3 or l_4 and w_2

LINDSTROM (1925) has shown that plants heterozygous for l_2 and w_1, w_2 or w_3 give ratios of 9 green, 3 yellow and 4 white seedlings in F_2 when self-pollinated. The segregations of L_2l_2 and W_2w_2 are modified by the linkage between these two factors. Genes l_3 and l_4 have been crossed with w_2 and give similar interactions. In the case of the cross $L_4l_4 \times W_2w_2$ the 9:3:4 ratio also is modified by linkage.

Trihybrid ratios involving l_1, i_j and l_2, l_3 or l_4

When plants of the genetic composition $L_1l_1L_3l_3I_ji_j$ were self-pollinated and their progenies grown the following classes of seedlings resulted.

27 $L_1L_4\ I_j$	green
9 $l_1L_4\ I_j$	green
9 $L_1L_4\ i_j$	white-iojap
9 $L_1l_3\ I_j$	yellow
3 $l_1l_3\ I_j$	yellow
3 $l_1L_4\ i_j$	yellow-iojap
3 $L_1l_3\ i_j$	white-striped-yellow
1 $l_1l_3\ i_j$	pale-yellow-striped-yellow

The triple recessive class is difficult to distinguish from the pure-yellow seedlings but has been observed. In these seedlings the deep yellow due to l_3 is suppressed by the i_j factor which in turn allows the lighter l_1 yellow to develop.

Data on the progenies from 89 self-pollinated plants heterozygous for these three factors are presented in table 1.

The data in table 1 do not show a very close fit to the expected numbers. When all of the yellow seedling classes are grouped together the fit is much better but still is not very good. The poor fit, however, is due chiefly to the deficiency of l_3 yellow seedlings.

TABLE 1

Seedling progenies from self-pollinated plants of the composition $L_1l_1L_2l_2I_ii_i$.

PEDIGREE	NUMBER OF PROGENIES	SEEDING CLASSES					
		GREEN	WHITE-IOJAP	YELLOW-IOJAP	YELLOW	WHITE STRIPED-YELLOW	PALE-YELLOW-STRIPED-YELLOW
1811[1]	1	117	34	14	55	7	0
1812[1]	1	104	21	7	44	9	0
2975[1]	40	2051	558	138	750	71	5
2976[1]	29	1541	356	115	552	75	8
2980[2]	11	667	178	47	208	31	1
2981[2]	7	357	105	39	115	12	0
Totals	89	4837	1252	360	1724	205	14
Expected (*36:9:3:12:3:1*)		*4721*	*1180*	*393*	*1574*	*393*	*131*
Deviations		+116	+72	−33	+150	−188	−117
		χ^2=218.7			P=very small		
Rearranged Totals		4837	1252	360		1943	
Expected (*36:9:3:16*)		*4721*	*1180*	*393*		*2098*	
Deviations		+116	+72	−33		−155	
		χ^2=21.47			P=0.0001		
Totals for F_2 progenies 71		3813	969	274	1401	162	13
Rearranged totals		3813	969	274		1576	
Expected (*36:9:3:16*)		*3731*	*933*	*311*		*1658*	
Deviations		+82	+36	−37		−82	
		χ^2=11.65			P=0.009		

[1] F_3 progenies from plants of the composition $L_1i_il_2l_2I_ii_iL_2$.

[2] F_3 progenies.

Data not included indicate that the progenies from plants heterozygous for $L_1l_1L_2l_2\ I_ii_i$ show similar interactions of the three factors but the ratios are modified by the linkage between l_1 and l_2 Progenies segregating for $L_1l_1L_4l_4\ I_ii_i$ have not been obtained but the indications are that such progenies would give similar interactions but that the ratios would be modified by the linkage between l_1 and l_4.

Trihybrid ratios involving l_2l_3, l_2l_4 *or* l_3l_4 *and* i_j

When plants of the composition $L_2l_2L_3l_3\ I_ii_i$ were self-pollinated their progenies gave the following genotypic and phenotypic classes of seedlings

27 $L_2L_3\ I_i$	green
9 $L_2L_3\ i_i$	white-iojap
9 $L_2l_3\ I_i$	yellow
9 $l_2L_3\ I_i$	
3 $l_2l_3\ I_i$	
3 $l_2L_3\ i_i$	white-striped-yellow
3 $L_2l_3\ i_i$	
1 $l_2l_3\ i_i$	

Data on the seedling progenies from 4 such self-pollinated plants are recorded in table 2. Because of the usual difficulty in classifying the white-striped-yellow seedlings, both of the yellow classes have been combined, thus making a 27:9:28 ratio of green:white-iojap:yellow seedlings. Excellent agreement between the observed numbers and those expected on the basis of independent inheritance was obtained, P having a value of 0.36.

TABLE 2

Seedlings progenies from self-pollinated plants of the composition $L_2l_2L_3l_3I_ii_i$.

PEDIGREE	NUMBER OF PROGENIES	GREENS	WHITE IOJAPS	YELLOWS	WHITE-STRIPED YELLOWS
4603	4	355	108	285	47
Rearranged totals		355	108	332	
Expected (27:9:28)		*335*	*112*	*348*	
Deviations		+20	−4	−16	

$\chi^2=2.07$ P=0.36

Other data show that similar ratios are obtained when plants of the composition $L_3l_3L_4l_4\ I_ii_i$ are self-pollinated. When plants of the composition $L_2l_2L_4l_4\ I_ii_i$ are self-pollinated the ratios are modified by the linkage between l_2 and l_4.

LINKAGE RELATIONS OF l_3

Linkage tests have been made between l_3 and factors in the R_a-G_{11},Y-P_1, B-L_g and R-G linkage groups. The data so far obtained indicate that l_3 is not located in any of these groups.

Tests of l_3 and i_i

Data already have been presented on l_3 and i_i in connection with the dihybrid $L_3l_3\ I_ii_i$ and the trihybrids $L_1l_1L_3l_3\ I_ii_i$ and $L_2l_2L_3l_3\ I_ii_i$. There was poor agreement between the numbers observed and those expected on the basis of indendepent inheritance for the first two of these segregations.

For the third segregation, however, P had a value of 0.37 indicating the independent inheritance of l_2, l_3 and i_j.

The ratios for the dihybrid segregation of $L_3l_3\ I_ji_j$ were distorted by deficiencies in the recessive classes. Crossover percentages computed from such distorted ratios would be of doubtful value and so are not included. The trihybrid $L_1l_1L_3l_3\ I_ji_j$, however, probably is worthy of more critical examination. The crossover percentages between l_3 and i_j have been computed for this material.

In the progenies from the trihybrid $L_1l_1L_3l_3\ I_ji_j$ there appeared to be no linkage between l_3 and i_j. Seventy-one F_2 progenies were used for studying any possible linkage between these two factors. Disregarding the segregation for l_1 the following numbers of seedlings were observed and expected:

	OBSERVED	*Expected*
green	3813	*3730*
iojap	1243	*1244*
yellow	1401	*1244*
striped-yellow	175	*414*

YULE'S coefficient of association and OWEN'S (1928) tables showed 37 percent crossing over in the repulsion phase. This situation does not agree with the manner in which the cross was made. There is an excess in the yellow seedling class and a large deficiency in the striped-yellow seedling class. This is due in part to the difficulty of distinguishing the pure-yellow seedlings and the yellow-striped-yellow seedlings. If these two classes are combined a 9:3:4 ratio results. The expected and observed numbers on the basis of this ratio are:

	OBSERVED	*Expected*
green seedlings	3813	*3730*
iojap seedlings	1243	*1244*
yellow seedlings	1576	*1658*

For this distribution χ^2 is 5.90 and P is 0.05. This is not a very close fit but there is no evidence of linkage. The crossover percentage as determined by a modification of EMERSON'S (1916) formula suggested by COLLINS (1924) was 49.

Probably the most critical data indicating the independent inheritance of l_3 and i_j come from a study of observed and expected numbers of the different kinds of F_3 progeny segregations from the self-fertilized normal green plants in some of the F_2 progenies mentioned above. The observed and expected numbers of F_3 seedling segregations from such plants, together with the corresponding genotype of the parental F_2 plants are recorded below.

Genotypes of the normal green F_1 plants	F_2 seedling segregation	Observed	*Expected*
1 $L_3L_3\ I_jI_j$	all green	9	*11*
2 $L_3l_3\ I_jI_j$	3 green:1 yellow	26	*21*
2 $L_3L_3\ I_ji_j$	3 green:1 iojap	25	*21*
4 $L_3l_3\ I_ji_j$	9 green:3 iojap:3 yellow: 1 striped-yellow	35	*42*

χ^2 for the above distribution is 3.48 and P is 0.34, showing very good agreement between the segregations observed and those expected on the basis of independent inheritance. From this evidence and that previously presented it seems safe to conclude that l_3 and i_j do not belong in the same linkage group. Unpublished data from recent investigations indicate that i_j is linked with ramosa and glossy 1. This would preclude l_3 from the $R_a\ G_{11}$ linkage group.

Tests of l_3 with y

Evidence from a study of seedling ratios and the proportions of various kinds of F_3 segregating progenies shows that l_3 and y, a factor for endosperm color, are independently inherited. No F_2 ratios are available but a summary of 11 F_3 seedling progenies is given in table 3.

TABLE 3

F_2 seedling progenies from self-pollinated plants of the composition $L_3l_3\ Yy$.

PEDIGREE	NUMBER OF PROGENIES	YELLOW ENDOSPERM		WHITE ENDOSPERM	
		NON-YELLOW SEEDLINGS	YELLOW SEEDLINGS	NON-YELLOW SEEDLINGS	YELLOW SEEDLINGS
2981	11	855	282	304	107
Expected 9:3:3:1		*871*	*290*	*290*	*97*
Deviations		−16	−8	+14	+10

$\chi^2=2.22$ P=0.53

The segregation agrees excellently with the expected 9:3:3:1 ratio, χ^2 being 2.22 and P being 0.53. It is realized that a summation of several F_3 progenies would obscure a loose linkage as some of the progenies might exhibit coupling and others repulsion and when summed the two conditions would tend to balance each other. A critical examination of the individual progenies to determine whether any of them exhibited either the coupling or repulsion phases of linkage between l_3 and y revealed several progenies in which coupling or repulsion might have existed. These progenies, however, deviate considerably from the expected 3 to 1 ratios for yellow and

white endosperm, and for green and yellow seedlings, preventing an accurate determination of linkage and suggesting that causes other than linkage were operating to distort the ratios for these particular characters.

Additional evidence from a study of the respective numbers of the several kinds of F_3 progenies from self-fertilized green F_2 plants offered further verification that l_3 and y were not linked. The observed and expected numbers of F_3 endosperm and seedling segregations from such plants, together with the corresponding genotypes of the parental F_2 plants are recorded below.

Genotypes of the Green F_3 plants	F_3 endosperm and seedling segregations	Observed	*Expected*
1 L_3L_3YY	All green from yellow seeds	4	*3.1*
2 L_3L_3Yy	Green seedlings, 3 yellow:1 white seed	7	*6.2*
2 L_3l_3YY	Yellow seeds, 3 green:1 yellow seedling	4	*6.2*
4 L_3l_3Yy	Segregating for both endosperm and seedling colors	16	*12.4*
1 L_3L_3yy	All green from white seeds	2	*3.1*
2 L_3l_3yy	3 green:1 yellow from white seeds	4	*6.2*

χ^2 for this distribution is 3.36 and P is 0.65, indicative of excellent agreement between the numbers obtained and those expected on the basis of the independent inheritance of l_3 and y. Gene l_3, therefore, probably is not located in the Y-P_1 linkage group.

Tests of l_3 with l_g

The linkage relations of l_3 and l_g, a simple recessive factor for liguleless leaves, was studied in the F_2 progenies from the cross $L_3l_3l_gl_g \times L_3l_3L_gL_g$. Seedling progenies were grown from 13 self-pollinated plants heterozygous for these two factors. The data on these progenies are summarized in table 4.

Some difficulty was experienced in classifying the yellow seedlings for the liguleless character. Ordinarily these seedlings were smaller than the non-yellow seedlings in the same progenies and the ligules were not well developed. For this reason the two groups of yellow seedlings have been combined, resulting in a 9:3:4 ratio.

Data on the F_2 progenies indicated that l_3 and l_g were not linked and as none of the F_3 progenies seemed to indicate linkage between these two factors the data from the F_2 and the F_3 progenies have been combined. For the F_2 progenies P was 0.13 and for all progenies combined P was 0.49.

Additional evidence that l_3 and l_g were not in the same linkage group came from a comparison of the actual numbers of the various kinds of F_3 progenies with the numbers expected on the basis of independent inheri-

TABLE 4

Seedling progenies from self-pollinated plants of the composition $L_3l_3L_gl_g$.

PEDIGREE	NUMBER OF PROGENIES	NON-YELLOW SEEDLINGS		YELLOW SEEDLINGS	
		NORMAL	LIGULELESS	NORMAL	LIGULELESS
1812[1]	1	106	26	36	17
2976[1]	12	573	233	161	114
2981[2]	8	630	207	194	74
Totals (F_2 Progenies)		679	259	197	131
Totals rearranged		679	259		328
Expected (9:3:4)		*712*	*237*		*316*
Deviations		−33	+22		+12
χ^2=4.03				P=0.13	
Totals (all progenies) 21		1309	466	391	205
Totals rearranged		1309	466	596	
Expected (9:3:4)		*1334*	*445*	*593*	
Deviations		−25	+21	+3	
χ^2=1.48				P=0.49	

[1] F_3 progenies from plants of the composition $l_3L_gl_3l_g$.

[2] F_3 progenies.

tance. The self-pollinated green F_2 phenotypes should segregate in the F_3 as shown:

Genotypes of the Green F_2 plants	F_3 seedling segregations	Observed	Expected
1 $L_3L_3L_gL_g$	All green	2	*3.1*
2 $L_3L_3L_gl_g$	3 green:1 liguleless green	8	*6.2*
2 $L_3l_3L_gL_g$	3 green:1 yellow	8	*6.2*
4 $L_3l_3L_gl_g$	9 green:3 liguleless green:3 yellow:1 liguleless yellow	9	*12.4*
1 $L_3L_3l_gl_g$	All liguleless green	4	*3.1*
2 $L_3l_3l_gl_g$	3 liguleless green:1 liguleless yellow	6	*6.2*

The value of χ^2 for the distribution above was only 2.51 and P was 0.77. This is an exceptionally close fit and indicates that l_3 does not belong in the *B*-L_g linkage group.

Tests of l_3 with genes in the R-G linkage group

Gene l_3 also has been tested for linkage with the following 4 factors

located in the *R-G* linkage group, l_1, l_2, l_4, and R^g. The tests with each of these factors have indicated that l_3 is not located on this chromosome.

l_3 and l_1

Possible linkage relations between l_3 and l_1 may be studied in the F_2 progenies from the trihybrid $L_1l_1L_3l_3I_ii_i$ presented in table 1 and already discussed. The observed numbers do not fit closely those expected on the basis of independent inheritance but the poor fit appears to be due to causes other than linkage between these two factors.

l_3 and l_2

A summary of the data on 6 F_2 progenies from plants of the genetic composition $l_2L_3L_3l_3$ is given below:

	Observed	*Expected*	Deviation
Non-yellow seedlings	724	*687*	+37
Yellow seedlings	498	*535*	−37

The deviation from the 9:7 ratio expected on the basis of independent inheritance is 3.1 times its probable error. The deviation, however, is in the wrong direction to indicate linkage. There is a large deficiency of yellow seedlings whereas there should have been an excess of yellow seedlings if these two factors were linked as they came into the cross from opposite parents.

l_3 and l_4

Gene l_4 is located in the *R-G* linkage group as will be shown by data to be presented later. Data are available on 9 progenies of F_1 plants of the composition $L_3l_4l_3L_4$. The data on these progenies are summarized below:

	Observed	*Expected*	Deviation
Green seedlings	921	*899*	+22
Yellow seedlings	677	*699*	−22

There is fairly close agreement between the observed and the expected numbers. Dev. ÷ P. E. is 1.7. Here, again, the deviations are in the wrong direction to indicate linkage.

l_3 and R^g

Genes l_1 and R^g are linked very closely, if not completely. As l_3 appeared to be independent of l_1 it naturally would be expected to be independent of R^g. Such provedto be the case. The data on l_3 and R^g come from 15 F_2

progenies from plants of the composition $L_3R^gl_3r^r$. A summary of the data on these progenies is given below.

	Observed	*Expected*	Deviation
L_3r^r—Non-yellow seedlings with red stems	757	*774*	−17
L_3R^g—Non-y ellow seedlings with green stems	276	*258*	+18
l_3r^r—Yellow seedlings with red stems	221	*258*	−37
l_3R^g—Yellow seedlings with non-red stems	122	*86*	+36

The excess of yellow seedlings with non-red stems is due to the difficulty of distinguishing red stem color on the small yellow seedlings. If all of the yellow seedlings are grouped together much better agreement is obtained between the expected and observed numbers. The results with this grouping, which gives a 9:3:4 ratio, are as follows:

	Observed	*Expected*	Deviation
Non-yellow seedlings with red stems	757	*774*	−17
Non-yellow seedlings with green stems	276	*258*	+18
All classes of yellow seedlings	343	*344*	−1

P is 0.46 for the above distribution, indicating very close agreement between the numbers observed and those expected on the basis of independent inheritance.

Tetrahybrid $L_1l_1L_3l_3I_iL_gl_g$

In the linkage studies with l_3 a number of interesting trihybrid and tetrahybrid segregations were obtained and it was thought worth while to report one of them. When plants heterozygous for $L_1l_1L_3l_3I_ii_iL_gl_g$ are self-pollinated the following genotypic and phenotypic classes of seedlings should result:

81	$L_1L_3I_iL_g$	green
27	$l_1L_3I_iL_g$	green
27	$L_1L_3I_il_g$	liguleless-green
27	$L_1l_3I_iL_g$	yellow
27	$L_1L_3i_iL_g$	white-iojap
9	$l_1l_3I_iL_g$	yellow
9	$l_1L_3i_iL_g$	yellow-iojap
9	$l_1L_3I_il_g$	liguleless-green
9	$L_1l_3i_iL_g$	white-striped-yellow
9	$L_1l_3I_il_g$	liguleless-yellow
9	$L_1L_3i_il_g$	liguleless-white-iojap
3	$l_1l_3i_iL_g$	pale-yellow-striped-yellow
3	$l_1l_3I_il_g$	liguleless-yellow
3	$L_1l_3i_il_g$	liguleless-white-striped-yellow
3	$l_1L_3i_il_g$	ligueless-yellow-iojap
1	$l_1l_3i_il_g$	liguleless-pale-yellow-striped-yellow

Assuming independent inheritance of these four factors, the various phenotypic seedling classes and the observed and expected numbers from four progenies are tabulated below:

Ratio	Phenotypes	Observed	*Expected*
108	green	137	*151*
36	liguleless-green	60	*50*
36	yellow	44	*50*
27	white-iojap	29	*39*
12	liguleless-yellow	46	*17*
9	liguleless-white-iojap	9	*13*
9	yellow-iojap	14	*13*
9	white-striped-yellow	7	*13*
3	liguleless-yellow-iojap	7	*4*
3	liguleless-white-striped-yellow	1	*4*
3	pale-yellow-striped-yellow	3	*4*
1	liguleless-pale-yellow-striped-yellow	2	*1*

The above distribution even though in fairly good agreement with the expected does not lend itself to statistical analysis because of certain deviations in the yellow-seedling classes which are relatively large, owing, perhaps, to difficulties in correctly classifying striped-yellow and liguleless-yellow seedlings.

If all of the yellow seedling types, exclusive of yellow-iojap, are combined, much better agreement between the actual and calculated numbers is obtained. These results are shown below:

Ratio	Phenotypes	Observed	*Expected*	Deviation
108	green	137	*151*	−14
36	liguleless-green	60	*50*	+10
27	white-iojap	29	*39*	−10
9	liguleless-white-iojap	9	*13*	− 4
9	yellow-iojap	14	*13*	+ 1
3	liguleless-yellow-iojap	7	*4*	+ 3
64	yellow seedlings of all classes	103	*90*	+13

The size of χ^2 for the above distribution was 11.30 and the value of P, 0.08. This fit, although not extremely good, tends to verify the hypothesis of independent inheritance of the four factors, $l_1 l_3 i_j l_9$.

LINKAGE RELATIONS OF l_4

Linkage tests with l_4 have shown that this factor is located in the *R-G* linkage group. Linkage data have been obtained with three factor pairs of this group, namely Rr, $L_2 l_2$ and $W_2 w_2$.

Tests of l_4 with R^g

Determinations of the linkage between l_4 and R^g come from data on a number of F_2 and F_3 seedling progenies from plants heterozygous for these two factors. The factor R^g is expressed in the seedlings as a green or at least a non-red stem, whereas seedlings carrying the factor r^r have red stems (provided, of course, that A is present).

Data on 6 progenies showing the repulsion phase of the linkage and 4 progenies showing the coupling phase of the linkage are recorded in table 5.

TABLE 5

Data on the progenies from self-pollinated plants of the composition $L_4l_4r^rR^g$.

PEDIGREE	RED STEMS		NON-RED STEMS	
	GREEN SEEDLINGS	YELLOW SEEDLINGS	GREEN SEEDLINGS	YELLOW SEEDLINGS
		Repulsion		
4606– 7	66	20	38	3
–11	152	65	62	10
5773– 4	114	36	43	5
5775–17	67	24	31	1
5776–14	103	50	36	11
–16	110	44	37	9
Totals	612	239	247	39
Expected, 37 percent crossing over	*607*	*245*	*245*	*39*
Deviation	+5	−6	+2	0
	$\chi^2=0.20$		P=very good fit	
		Coupling		
5773–34	46	13	7	3
–41	37	12	7	3
5775–39	209	78	66	35
5776–37	179	11	21	27
Totals	471	114	101	68
Expected, 36 percent crossing over	*454*	*111*	*111*	*77*
Deviation	+17	+3	−10	−9
	$\chi^2=2.67$		P=0.45	

The 6 repulsion progenies showed 37 percent crossing over and the 4 coupling progenies 36 percent crossing over. These two crossover percentages are in very close agreement.

Three of the repulsion progenies recorded in table 5 were F_2 progenies segregating for aleurone color. The genetic composition of the parental plants was L_4R^gC/l_4r^rc. The progenies from these plants showed 3:1

ratios of red to non-red stems and 9:7 ratios of colored to colorless aleurone. The repulsion phase of the linkage was exhibited by the stem color segregation and the coupling phase by the aleurone segregation. This peculiar situation is due to the action of the factor R^g which is dominant in aleurone color and recessive in stem color. LINDSTROM (1925) described a similar situation in discussing the linkage of L_2 and R^g. The complete data on these three F_2 progenies are recorded in table 6.

TABLE 6

Data on the F_2 progenies from plants of the composition L_4R^gC/l_4r^gc.

PEDIGREE	COLORED ALEURONE				COLORLESS ALEURONE			
	RED STEMS		NON-RED STEMS		RED STEMS		NON-RED STEMS	
	L_4	l_4	L_4	l_4	L_4	l_4	L_4	l_4
4606– 7	41	9	31	2	25	11	7	1
–11	78	13	42	6	74	52	20	4
5775–17	37	13	18	1	30	18	6	0
Totals	156	35	91	9	129	81	33	5

A summary of the data in table 6 to show the repulsion phase of the linkage between L_4l_4 and r^gR^g appears below:

	L_4r^g	L_4R^g	l_4r^g	l_4R^g
Observed	285	124	116	14
Expected, 32 percent crossing over	*283*	*121*	*121*	*14*
Deviations	+2	+3	−5	0
$\chi^2=0.30$			P=very good fit	

Another summary of these data to show the coupling phase of the linkage between these two factor pairs appears below:

	Colored Aleurone		Colorless Aleurone	
	L_4	l_4	L_4	l_4
Observed	247	44	162	86
Expected, 30 percent crossing over	*252*	*51*	*152*	*84*
Deviations	−5	−7	+10	+2
$\chi^2=1.77$			P=0.62	

These data indicate a modified 27:9:21:7 ratio. The crossover percentage was computed using a modification of EMERSON's (1916) general formula described by BRUNSON (1924) for use in trihybrid ratios involving two complementary genes, one of which is linked with a third gene.

The crossover percentages of 32 and 30 computed from these two distributions are slightly lower than those of 36 and 37 computed from the data in table 5. The value of 36 percent has been selected as the most likely value as it is based on coupling progenies and also because it fits better than the lower values certain data presented later in tables 11, 12, 13 and 15.

Tests of l_4 with w_2

The data on the linkage relations between l_4 and w_2 come from 77 F_3 seedling progenies of the cross $L_4L_4W_2w_2 \times L_4l_4W_2W_2$. In as much as these were F_3 progenies, some of them showed the coupling phase of the linkage and others the repulsion phase. The summarized data on these progenies are recorded in table 7.

TABLE 7

Data on the F_3 seedling progenies exhibiting the coupling and the repulsion phases of linkage between l_4 and w_2.

PEDIGREE	NUMBER OF PROGENIES	GREEN SEEDLINGS	YELLOW SEEDLINGS	WHITE SEEDLINGS
		Coupling		
5773	4	396	86	126
5774	7	667	147	253
5775	14	1465	375	569
5776	6	689	164	248
Totals	31	3217	772	1196
Expected, 35 percent crossing over		*3140*	*749*	*1296*
Deviation		+77	+23	−100
	$\chi^2=10.21$		P=0.006	
		Repulsion		
5773	16	1298	542	563
5774	8	425	169	177
5775	17	1720	658	690
5776	5	485	167	176
Totals	46	3928	1536	1606
Expected, 40 percent crossing over		*3818*	*1485*	*1766*
Deviation		+110	+51	−160
	$\chi^2=21.84$		P=0.00002	

The crossover percentages were computed from a modification of EMERSON'S formula suggested by COLLINS (1924) for use with a 9:3:4 ratio. The data in table 7 indicate 35 percent crossing over between l_4 and w_2 for the coupling progenies and 40 percent for the repulsion progenies.

The poor fit in both the coupling and the repulsion progenies is due largely to the deficiency of white seedlings. This deficiency results from the very close linkage between w_2 and a factor for defective seeds, d_f. This linkage was reported by LINDSTROM (1923). The defective seeds germinated only 91 percent, whereas the normal seeds gernimated 97 percent.

An opportunity to verify the crossover percentages computed from the seedling ratios presented itself in a study of the proportions of the different kinds of F_3 segregations. It will be recalled that l_4 and w_2 came into the cross from opposite parents and the repulsion phase of the linkage would be expected in F_2. The closeness of the linkage between these two genes will be reflected in the proportions of the various kinds of F_3 progenies from self-pollinated F_2 green plants. The theoretical F_2 populations were computed assuming 35, 40 and 44 percent crossing over respectively and the proportions of the different kinds of F_3 segregations determined.

Table 8 shows a comparison of the observed and expected numbers of F_3 progeny segregations. A total of 146 F_3 progenies is available for study. The χ^2 test for goodness of fit has been determined between the observed numbers of F_3 segregations and those expected with 35, 40 and 44 percent crossing over. The best fit was obtained when 35 percent crossing over was assumed, although each of the crossover percentages given fits well within the limits of chance variations, P in the case of 35 percent crossing over being greater than 0.80.

TABLE 8

Observed numbers of the different kinds of F_3 progeny segregations from F_1 plants of the composition Lw_2/l_4W_2 and those expected with 35, 40, and 44 percentages of crossing over.

CROSSOVER PERCENTAGE	F_3 SEEDLING SEGREGATIONS			
	BREEDING TRUE FOR GREEN	SEGREGATING FOR 3 GREEN: 1 WHITE	SEGREGATING FOR 3 GREEN: 1 YELLOW	SEGREGATING FOR 9 GREEN: 3 YELLOW: 4 WHITE[1]
Observed	8	28	33	77
Expected, 35 percent crossing over	*8*	*31*	*31*	*75*
	$\chi^2=0.47$		P=greater than 0.80	
Observed	8	28	33	77
Expected, 40 percent crossing over	*11*	*32*	*32*	*70*
	$\chi^2=2.05$		P=0.56	
Observed	8	28	33	77
Expected, 44 percent crossing over	*13*	*33*	*33*	*66*
	$\chi^2=4.51$		P=0.22	

[1] Modified by the linkage between l_4 and w_2.

Tests of l_4 with l_2

The percentage of crossing over between l_4 and l_2 was determined from the F_2 seedling progenies of the cross $L_2l_2L_4L_4 \times L_2L_2L_4l_4$. The complementary interaction of these two factors produced a 9:7 ratio of green to yellow seedlings modified slightly by linkage. The summarized data on 10 F_2 progenies are recorded in table 9.

TABLE 9

F_2 seedling progenies from self-pollinated plants of the composition L_2l_4/l_2L_4.

PEDIGREE	NUMBER OF PROGENIES	SEEDLING CLASSES		DEV ÷ P.E. 9:7
		GREEN	YELLOW	
5770 5771	10	1156	963	
Expected, 9:7		*1192*	*927*	
Deviation		−36	+36	2.4
Expected, 43 percent crossing over		*1157*	*962*	
Deviation		−1	+1	0.7

The data in table 9 indicate 43 percent crossing over between l_2 and l_4. The deviation from the numbers expected on the basis of independent inheritance is only 2.4 times the probable error. The deviation however is in the direction of an excess of yellow seedlings which would be expected if the two factors were linked. In view of the usual deficiences obtained in the yellow seedling classes an excess in this case is of added significance.

Recognition that any deficiency of yellow seedlings would tend to increase the percentage of crossing over when calculated by the formula used (a modification of EMERSON'S formula for 9:7 ratios) would suggest, perhaps, that 43 percent was slightly high. Because of that possibility, it was assumed that all kernels not germinated were potentially yellow seedlings. The ratio then became 1156 green and 1012 yellow seedlings from which 37 percent of crossing over was calculated. This value represented the lowest possible percentage of crossing over obtainable from the data at hand, indicating that l_2 and l_4 were linked weakly even by the most conservative estimation.

Forty-three percent crossing over between l_4 and l_2 was decided upon as being more nearly correct. This figure agreed remarkable well with the calculations of crossover values between other factors within the linkage group.

THE LINKAGE GROUP L_4-L_1, $R-W_2-L_2$

LINDSTROM (1925) indicated that the probable arrangement of the genes he studied was $L_1,R-W_2-L_2$. L_1 and R probably are completely linked, and he obtained about 22 percent crossing over between L_1 and W_2 and about 35 percent crossing over between R and L_2. Data already presented in this paper indicate that L_4 is about 36 units from R, 35 to 40 units from W_2 and 43 units from L_2. This situation would indicate that L_4 probably is located to the left of R, although if this is the case the distances from L_4 to R and from L_4 to W_2 do not seem to be in very close agreement.

The best proof that this is the real situation could, of course, be had from backcrosses involving l_4 and l_2 or more of the other factors on this chromosome. In as much as 3 of the genes studied (l_2 l_4 and w_2) are lethal, backcrosses were not possible. The best available proof that this is the correct order of these genes comes from data on 2 trihybrid segregations, one involving l_4, R^g and w_2 and the other involving l_4, R^g and l_2.

The trihybrid $L_4l_4r^rR^gW_2w_2$

This trihybrid was the result of the cross $L_4L_4r^rR^gW_2w_2 \times L_4l_4r^rr^rW_2W_2$. The F_1 plants of this cross would be expected to be of 8 different genotypes $L_4r^rW_2/L_4r^rW_2$, $l_4r^rW_2/L_4r^rW_2$, $L_4r^rW_2/L_4r^rw_2$, $l_4r^rW_2/L_4r^rw_2$, $L_4r^rW_2/L_4R^gW_2$, $l_4r^rW_2/L_4R^gW_2$, $L_4r^rW_2/L_4R^gw_2$, $l_4r^rW_2/L_4R^gw_2$. Twelve F_1 plants were self-pollinated. Three of these plants proved to be of the genotype, $l_4r^rW_2/L_2R^gw_2$. The F_2 progenies from two of them were grown in the field and 135 $L_4r^rW_2$ plants (green plants with red stems) were self-pollinated. Some of these plants proved to be homozygous for all 3 of the factors involved, some were heterozygous for 1, some for 2 and some for all 3 of the factors. The F_3 progenies from plants heterozygous for all 3 genes (57 in all) furnish the most critical evidence that the order of the genes is $L_4-R^g-W_2$.

Four different genotypes were expected among the self-pollinated F_2 plants that were heterozygous for all 3 genes. These four genotypes and their F_3 linkage relations are shown below:

	F_2 genotype	F_3 linkage relations
1.	$\frac{l_4r^rW_2}{L_4R^gw_2}$	Repulsion between l_4 and R^g, and between l_4 and w_2 and coupling between R^g and w_2.
2.	$\frac{L_4r^rW_2}{l_4R^gw_2}$	Coupling between l_4 and R^g, between l_4 and w_2, and between R^g and w_2.
3.	$\frac{l_4r^rw_2}{L_4R^gW_2}$	Repulsion between l_4 and R^g, coupling between l_4 and w_2 and repulsion between R^g and w_2.
4.	$\frac{l_4R^gW_2}{L_4r^rw_2}$	Coupling between l_4 and R^g, repulsion between l_4 and w_2 and between R^g and w_2.

Each of the 57 progenies from trihybrid F_2 plants was examined to determine the genotype of the parental F_2 plant. Progenies were obtained representing the first three parental genotypes. If the arrangement of the genes is $L_4 - R^g - W_2$ the fourth parental genotype represents the double crossover combination of these three genes and would be expected to occur less frequently than the non-crossover or single-crossover combinations. No F_3 progenies representing this parental genotype were obtained.

A summary of the data on all of these progenies from which the crossover percentage between R^g and w_2 was computed is recorded in table 10. Data on the coupling progenies indicate 17 percent of crossing over between these two factors and data on the repulsion progenies indicate 13 percent of crossing over. Inasmuch as the numbers were much larger in the coupling progenies the value of 17 percent has been used in computing the expected numbers for the F_3 progenies of the different F_2 parental genotypes. The poor fit in the coupling progenies is due to the deficiency of white seedlings. This value is intermediate between that of 15.4 previously

TABLE 10

Data on the F_3 progenies from F_2 plants of the composition $r^r R^g W_3 w_3$.

PEDIGREE	NUMBER OF PROGENIES	NON-WHITE SEEDLINGS		WHITE SEEDLINGS	
		RED STEMS	NON-RED STEMS	RED STEMS	NON-RED STEMS
		Coupling			
5773	15	1540	178	139	385
5774	6	464	55	50	121
5775	28	3337	448	343	777
5776	4	507	81	45	128
Totals	53	5848	762	577	1411
Expected, 17 percent crossing over		*5780*	*669*	*669*	*1481*
Deviation		+68	+93	−92	−70
	$\chi^2 = 29.69$		P = very small		
		Repulsion			
5773	1	107	41	46	1
5774	2	201	98	92	0
5775	1	95	43	41	2
5776	1	91	57	45	1
Totals	5	494	239	224	4
Expected, 13 percent crossing over		*485*	*236*	*236*	*4*
Deviation		+9	+3	−12	0
	$\chi^2 = 0.82$			P = very good fit	

reported by LINDSTROM (1924) between the same two factors and that of 22 reported by him (1925) between L_1 and W_2.

Unfortunately it was difficult to compute the crossover percentage between l_4 and R^g due to the presence of w_2. The linkage between w_2 and genes l_4 and R^g resulted in unequal suppression of l_4 classes by w_2 and made any calculation of linkage between l_4 and R^g difficult and uncertain. For this reason the crossover value of 36 percent arrived at from the data in table 5 has been used in computing the expected numbers for the F_3 progenies of the different F_2 genotypes.

Assuming 36 percent crossing over between l_4 and R^g and 17 percent crossing over between R^g and w_2, a double crossover percentage of about 6 would be expected if there were no interference. The theoretically expected numbers have been computed on the basis of these crossover values.

The parental genotype $l_4 r^r W_2/L_4 R^g w_2$

With the crossover percentages mentioned above the relative frequency of the gametes produced by this parental combination would be as follows:

Gametic ratio	Gametes	Method of gamete formation
53	$L_4R^gw_2$	Parental type
30	$L_4r^rW_2$	Crossover in the L_4–R^g region
11	$L_4R^gW_2$	Crossover in the R^g–W_2 region
6	$L_4r^rw_2$	Double crossover
6	$l_4R^gW_2$	Double crossover
11	$l_4r^rw_2$	Crossover in the R^g–W_2 region
30	$l_4R^gw_2$	Crossover in the L_4–R^g region
53	$l_4r^rW_2$	Parental type

TABLE 11

Seedling progenies from self-pollinated plants of the composition $l_4r^rW_2/L_4R^gw_2$.

PEDIGREE	NUMBER OF PROGENIES	GREENS		YELLOWS		WHITES	
		RED STEMS	NON-RED STEMS	RED STEMS	NON-RED STEMS	RED STEMS	NON-RED STEMS
5773	13	901	131	401	22	113	341
5774	5	234	38	98	6	38	79
5775	17	1469	251	621	37	206	484
5776	1	92	22	36	3	11	33
Totals	36	2696	442	1156	68	368	937
Expected[1]		*2687*	*385*	*1122*	*56*	*441*	*976*
Deviation		+9	+57	−34	+12	−73	−39

$\chi^2 = 25.71$ P=0.0001

[1] Calculated on the basis that there was 36 percent crossing over between L_4 and R_g, 17 percent crossing over between R^g and W_2, and 6 percent of double crossing over.

By arranging these gametes in an ordinary trihybrid Punnett square and multiplying them by their relative frequencies, a theoretical phenotypic population can be derived, which should agree with the one observed, provided the linear order of the genes and their distances apart have been correctly assumed. In table 11 are summarized the results from 36 progenies. The agreement between the observed and expected numbers is not very satisfactory due to the deficiency of white seedlings. This deficiency is due to the linkage between w_2 and d_f.

The parental genotype $L_4 r^r W_2/l_4 R^g w_2$.

Assuming the same crossover percentages as before the gametic ratio for this parental combination would be as follows:

Gametic ratio	Gamete	Method of gamete formation
53	$L_4 r^r W_2$	Parental type
30	$L_4 R^g w_2$	Crossover in the L_4–R^g region
11	$L_4 r^r w_2$	Crossover in the R^g–W_2 region
6	$L_4 R^g W_2$	Double crossover
6	$l_4 r^r w_2$	Double crossover
11	$l_4 R^g W_2$	Crossover in the R^g–W_2 region
30	$l_4 r^r W_2$	Crossover in the L_4–R^g region
53	$l_4 R^g w_2$	Parental type

Data on 17 progenies from F_2 plants of this genotype are recorded in table 12.

TABLE 12

Seedling progenies from self-pollinated plants of the composition $L_4 r^r W_2/l_4 R^g w_2$.

PEDIGREE	NUMBER OF PROGENIES	GREENS		YELLOWS		WHITES	
		RED STEMS	NON-RED STEMS	RED STEMS	NON-RED STEMS	RED STEMS	NON-RED STEMS
5773	2	191	16	47	9	26	44
5774	1	103	9	29	2	12	42
5775	11	1031	93	216	67	137	293
5776	3	305	41	74	15	34	95
Totals	17	1630	159	366	93	209	474
Expected[1]		*1587*	*134*	*383*	*94*	*228*	*505*
Deviation		+43	+25	−17	−1	−19	−31

$\chi^2 = 10.08$ P = 0.07

[1] Calculated on the basis that there was 36 percent crossing over between L_4 and R^g, 17 percent between R^g and W_2 and 6 percent of double crossing over.

The fit in this case is much better, P being 0.07. There is a deficiency of white seedlings and an excess of green seedlings with non-red stems.

The parental genotype $l_4r^rw_2/L_4R^gW_2$

The expected gametic ratio for this parental combination, with the crossover percentages previously mentioned, would be as follows:

Gametic ratio	Gamete	Method of gamete formation
53	$L_4R^gW_2$	Parental type
30	$L_4r^rw_2$	Crossover in the L_4–R^g region
11	$L_4R^gw_2$	Crossover in the R^g–W_2 region
6	$L_4r^rW_2$	Double crossover
6	$l_4R^gw_2$	Double crossover
11	$l_4r^rW_2$	Crossover in the R^g–W_2 region
30	$l_4R^gW_2$	Crossover in the L_4–R^g region
53	$l_4r^rw_2$	Parental type

The theoretical numbers of each phenotype were calculated in a manner similar to that previously described.

This parental genotype was represented by only four seedling progenies. The data on these progenies are recorded in table 13. The agreement between the observed and the expected numbers was very close, P having a value of 0.54.

TABLE 13

Seedling progenies from self-pollinated plants of the composition $l_4r^rw_2/L_4R^gW_2$.

PEDIGREE	NUMBER OF PROGENIES	GREEN SEEDLINGS		YELLOW SEEDLINGS		WHITE SEEDLINGS	
		RED STEMS	NON-RED STEMS	RED STEMS	NON-RED STEMS	RED STEMS	NON-RED STEMS
5774	2	159	87	42	11	92	0
5775	1	69	37	26	6	41	2
5776	1	69	48	22	9	45	1
Totals	4	297	172	90	26	178	3
Expected[1]		*288*	*162*	*101*	*24*	*186*	*6*
Deviation		+9	+10	−11	+2	−8	−3

$\chi^2=4.11$ P=0.54

[1] Calculated on the basis that there was 36 percent crossing over between L_4 and R^g, 17 percent between R^g and W_2, and 6 percent of double crossing over.

With the crossover percentages previously mentioned (36 percent between l_4 and R^g, 35 to 40 percent between l_4 and w_2 and 17 percent between

R^g and w_2) there may be some doubt as to whether the order of these genes is $L_4-R^g-W_2$ or $R^g-W_2-L_4$. With only 17 percent crossing over between R^g and W_2 it seems extremely unlikely that the order could be $R^g-L_4-W_2$ With such an arrangement L_4 would have to be closely linked with both R^g and W_2 instead of loosely linked with them.

In order to determine which of the first two arrangements was the more probable the theoretical ratios expected with the order $R^g-W_2-L_4$ were computed for the data in tables 11, 12 and 13. The values of χ^2 then were computed and are shown in table 14 in comparison with the χ^2 values computed for the order $L_4-R^g-W_2$.

TABLE 14

The χ^2 values for the expected ratios in tables 11, 12, and 13 with the gene orders L_4–R^g–W_2 and R^g–W_2–L_4.

TABLE NUMBER	χ^2 VALUE WITH THE GENE ORDER INDICATED	
	L_4–R_g–W_2	R_g–W_2–L_4
11	25.71	64.70
12	10.08	43.31
13	4.11	4.27
Totals	39.90	112.28

The χ^2 values in table 14 indicate that the order $L_4-R^g-W_2$ is probably the correct arrangement of these genes.

The trihybrid $L_4l_4r^gR^gL_2l_2$

Additional information indicating that l_4 occupied a locus to the left of R is supplied by the F_2 progenies of the cross $L_4L_4r^gR^gL_2l_2 \times L_4l_4r^gr^gL_2L_2$. The F_1 plants of this cross were of several different genotypes. Those heterozygous for all three factors would be represented by the genetic formula $l_4r^gL_2/L_4R^gl_2$. The F_2 progenies from these plants would show coupling between R^g and l_2 and repulsion between l_4 and R^g and between l_4 and l_2.

It was not possible to compute the crossover percentages between L_4 and R and between L_2 and R from these progenies. Therefore, the value of 36 percent previously determined was assumed between L_4 and R^g and that of 35 percent reported by LINDSTROM (1925) was assumed between L_2 and R^g. On the basis of these two assumed values, and the order

$L_4 - R^g - L_2$, about 13 percent of double crossing over would be expected. The expected gametic ratios, therefore, would be as follows:

Gametic ratio	Gametes	Method of Gamete formation
42	$L_4R^gl_2$	Parental type
22	$L_4R^gL_2$	Crossover in the R^g–L_2 region
23	$l_4R^gl_2$	Crossover in the L_4–R^g region
13	$L_4{}^gl_2$	Double crossover
13	$l_4R^gL_2$	Double crossover
23	$L_4{}^gL_2$	Crossover in the L_4–R^g region
22	$l_4{}^gl_2$	Crossover in the R^g–L_2 region
42	$l_4{}^gL_2$	Parental type

The factorial interaction of this trihybrid produced a modified 27:9:21:7 ratio of greens with red stems, greens with non-red stems, yellows with red stems and yellows with non-red stems. A comparison of the observed numbers of seedlings in each of these phenotypic classes with the expected numbers calculated according to the gametic ratios assigned is shown in table 15.

TABLE 15

Seedling progenies from self-pollinated plants of the genetic composition $l_4{}^gL_2/L_4R^gl_2$.

PEDIGREE	NUMBER OF PROGENIES	GREENS		YELLOWS	
		RED STEMS	NON-RED STEMS	RED STEMS	NON-RED STEMS
5770 5771	5	423	112	303	112
Expected[1]		*404*	*119*	*308*	*119*
Deviation		+19	−7	−5	−7

$\chi^2 = 1.80$ P = 0.62

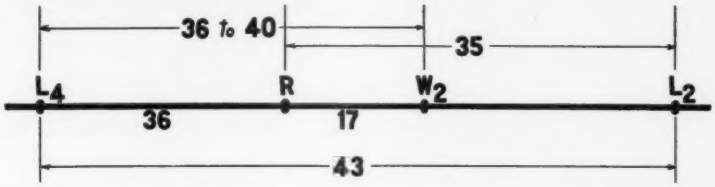
[1] Calculated on the assumption that there was 36 percent of crossing over between L_4 and R^g and 35 percent between R^g and L_2 and 13 percent of double crossing over.

The crossover percentages assumed for computing the expected distribution evidently must be fairly correct as the fit is very good, χ^2 being only 1.80 and P being 0.62.

CHROMOSOME MAP

According to the results obtained in this study, supplemented by work already done by LINDSTROM, who determined the $R - W_2 - L_2$ relationship,

the order of the four factors with their approximate crossover percentages is as shown in the diagram below:



The interrelations of these crossover percentages when the double crossover values are taken into account are in very close agreement. For example, the crossing over between L_4 and L_2 was 43 percent as determined from data involving these two factors only. Crossing over between L_4 and R, and R and L_2 was 36 and 35 percent, respectively, which would mean about 13 percent of double crossovers in the three factor relation L_4-R-L_2. As double crossing over reduces the actual amount determined between any two factors in a series, the total crossover percentage between L_4 and L_2 should be 43 percent plus twice 13 percent or 69 percent, which agrees very well with 71 percent, the sum of crossover percentages between L_4 and R, and R and L_2. Another three factor relation, L_4-R-W_2, furnished similar data. The double crossovers here were calculated to be about 6 percent as the percentages between L_4 and R, and R and W_2 were 36 and 17 percent, respectively. About 35 or 40 percent of crossing over existed between L_4 and W_2 to which amount should be added twice the double crossover percentage, making a total of 47 to 52 percent. The sum of the crossover percentages from L_4 to R and from R to W_2 is about 53 percent. If the correct distance from L_4 to R is 36 units probably the distance from L_4 to W_2 is at least 40 units or more, rather than 35.

SUMMARY

Two new lethal factors for yellow seedlings in maize, both simple recessives, are reported. The allelomorphic factor pairs are designated L_3l_3 and L_4l_4.

The interaction of these two genes with the two previously reported genes producing yellow seedlings and with certain other chlorophyll genes also is described. Genes l_3 and l_4 are similar to l_2 in their interactions with other chlorophyll factors.

The linkage group to which l_3 belongs was not determined. It showed no indication of linkage with certain members of the $R-G$, $B-L_9$, $Y-P_l$ and $Ra-G_{11}$ linkage groups.

Gene l_4 showed linkage with 3 members of the $R-G$ linkage group. Its most probable location appears to be 36 units to the left of R.

The order of the four genes of this linkage group included in this study appears to be $L_4-R-W_2-L_2$.

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